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We are studying brothers with and without prostate cancer in order to investigate the potential relation between genetic factors and their disease. During the reporting period of this Idea Development Award we have concluded recruitment, exceeding our sample size goals (over 1,000 men total recruited). From each of these men we have obtained 30 mls of blood and a risk factor questionnaire. Using these data, and in collaboration with other researchers, we have made four extremely encouraging discoveries with regard to the potential relation of genetic factors to prostate cancer occurrence and aggressiveness. First, using an association approach, we have detected a relation between a variant in the CYP3A4 gene and prostate tumor aggressiveness among African-Americans. Second, in a collaborative linkage analysis, we have undertaken two genome-wide scans that localized candidate regions possibly harboring genes for the development of prostate cancer. Third, we have undertaken a genome-wide linkage analysis of tumor aggressiveness genes, localizing regions that may harbor genes affecting prostate cancer progression. Finally, we have followed up our linkage results with loss of heterozygosity analyses that have substantially narrowed the width of the most promising candidate regions. Our results implicate genes in the development and/or aggressiveness of prostate cancer.

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Introduction

Prostate cancer is the most common non-skin cancer among men in the United States. This disease appears to run in families; many men who have close relatives with prostate cancer are at an increased risk of developing this disease themselves. Furthermore, such men might respond differently to treatment than men without a family history of prostate cancer. One possible explanation for the familiality of prostate cancer is genetics, and recent research has suggested some regions in DNA that might harbor alterations that increase the risk. We are studying brothers with and without prostate cancer in order to investigate the potential relation between genetic factors and their disease. During the current reporting period of this Idea Development Award we have concluded recruitment, exceeding our sample size goals (over 1,000 men total recruited). From each of these men we have obtained 30 mls of blood and a risk factor questionnaire. Using these data, and in collaboration with other researchers, we have made four extremely encouraging discoveries with regard to the potential relation of genetic factors to prostate cancer occurrence and aggressiveness, and published numerous articles describing presenting these findings to the scientific community. We introduce these findings below, and provide more complete details within the remainder of this report. In a collaborative linkage analysis, we have undertaken two genome-wide scans that localized candidate regions possibly harboring genes for the development of prostate cancer. We also have undertaken a genome-wide linkage analysis of tumor aggressiveness genes, localizing regions that may harbor genes affecting prostate cancer progression. Finally, we have followed up our linkage results with loss of heterozygosity analyses that have substantially narrowed the width of the most promising candidate regions. Our results implicate genes in the development and/or aggressiveness of prostate cancer. The information from this study should ultimately help provide men with additional knowledge about their risk of prostate cancer and, if they are already diseased, how genes might influence their response to treatment.

Body

Below we describe out research accomplishments with respect to the original Statement of Work. For focus and clarity we reproduce the approved tasks that were proposed for completion during the current reporting period. We then note our corresponding accomplishments, pointing out any issues arising during the course of this work.

Task 5. Genotype 400 subjects' blood

- **a. Extract DNA from blood samples.**
- **b. Amplify relevant candidate regions and genes.**
- **c. Enter genotypic information into database.**
- a. **Write up year 2 report and compete for Phase II funds.**

We have fully completed Task 5. We have extracted DNA from all of the study subject's samples. To date, we have amplified relevant candidate genes and regions in over 600 blood samples. The resulting information has been electronically entered into our database system. In lieu of the year 2 report, per U.S. Army Medical Research and Materiel Command instructions we wrote up a Phase

I 18-Month Competitive Progress Report. Finally, based on promising findings during the first 18 months of Phase I, we successfully competed for Phase II funds.

Task 6. Analyze data and present results

- **a. Check data for inconsistencies, ambiguities, and missing values (data quality control will be implemented throughout collection and genotyping).**
- **b. Undertake linkage and association analyses.**
- **c. Write up final report.**

We have also completed Task 6. We have cleaned and analyzed data arising from samples collected for this project. We have undertaken linkage and association analyses searching for prostate cancer genes. This work has resulted in important discoveries about the genetic basis of prostate cancer development and aggressiveness. First, we have collaborated on genome-wide linkage analyses searching for prostate cancer susceptibility genes using the sibling data (Suarez et al.2000a, attached as Appendix 1; Suarez et al., 2000b, attached as Appendix 2). This work detected five regions with nominally statistically significant linkages (i.e., $p<0.05$) on chromosomes 2q, 12p, 15q, 16p, and 16q. The strongest result was for the region on chromosome 16q23, with a peak near marker D16S3096 ($p<0.001$).

Using the same data as above, we have undertaken a novel genome-wide scan to map candidate regions for prostate cancer aggressiveness genes (Witte et al., 2000, attached as Appendix 3). Here we used Gleason score as a surrogate for tumor aggressiveness because it is generally considered a strong predictor of survival with prostate cancer. For our statistical analysis we used a linear regression approach whereby the mean-corrected cross product between brothers' Gleason scores is regressed on the estimated proportion of marker alleles shared among brothers identical-by-descent. We found strong evidence for linkage with Gleason score in four genomic regions on chromosomes 5, 7, 10, and 19, with low p-values ($p<0.01$) extending across relatively broad regions on these chromosomes.

We followed up these linkage results with association and loss of heterozygosity (LOH) studies focused on delineating more precisely the linkage regions. In particular, we obtained biospecimens from 51 men, and studied LOH at microsatellite markers in the linkage peak regions (some of which were included in the original sib-pair analysis). We detected substantial LOH across many of these markers, with a peak of 53% LOH at marker D16S516 (Paris et al., 2000, attached here as Appendix 4). Furthermore, thirty-seven of the 51 specimens studied (72%) showed LOH that involved markers D16S3096 and/or D16S516. Focusing on these two markers, 17 of the 51 specimens (33%) showed specific regional loss, narrowing the potential candidate region down to approximately 120 kb in width. Another promising region of LOH was detected on chromosome 7q, a region where we detected linkage with disease aggressiveness (Neville et al., in preparation). As Part of our Phase II project, we are presently using contig development, expressed sequence tagged (EST) mapping, and solution hybrid capture in an attempt to clone the putative prostate cancer susceptibility genes residing within these regions.

Key Research Accomplishments

During the current reporting period of this grant we have produced the following key research accomplishments.

- Completed recruitment (of over 519 men) into the study. That is, we have collected and stored consent, blood, and questionnaire information on these men.

- Undertaken extremely promising linkage and association analyses. Results from this work provide strong evidence for the existence of prostate cancer susceptibility and aggressiveness loci. A number of publications have arisen from this work, and we continue to pursue the potential genes residing within these regions.

Reportable Outcomes

During this reporting period, our reportable outcomes include the following manuscripts, presentations, and grant received (based in part on extending our results to a new study population).

Manuscripts

- Suarez BK, Lin J, Burmester JK, Broman K, Weber JL, Banerjee TK, Goddard KA, Witte JS, Elston RC, Catalona WJ. A genome screen of multiplex sibships with prostate cancer. American Journal of Human Genetics 2000;66:933-944.
- Witte JS, Goddard KAB, Conti DV, Elston RC, Lin J, Suarez BK, Broman KW, Burmester JK, Weber JL, Catalona WJ. Genome-wide scan for prostate cancer aggressiveness loci. American Journal of Human Genetics 2000;67:92-99.
- Paris PL, Witte JS, Kupelian PA, Levin H, Klein EA, Catalona WJ, Casey G. Identification and fine mapping of a region showing a high frequency of allelic imbalance on chromosome 16q23.2 that corresponds to a prostate cancer susceptibility locus. Cancer Research 2000;60:3645-3649.
- Suarez BK, Lin J, Witte JS, Conti DV, Resnick MI, Klein EA, Burmester JK, Vaske DA, Banerjee TK, Catalona WJ. Replication linkage study for prostate cancer susceptibility genes. Prostate 2000;45:106-114.

Presentations

- Genetic epidemiology of prostate tumor aggressiveness, Case Western Reserve University Blood Club / Cancer Center Seminar, November 1999.
- Prostate cancer genetic epidemiology, Case Western Reserve University / University Hospitals Ireland Cancer Center Trainees Meeting, December 1999.
- Genetic epidemiology of prostate cancer, American Cancer Society: Man-to-Man, Cleveland, April 2000.
- Localization of Prostate Cancer Genes: from Linkage to Characterization, School of Public Health, University of Michigan, November 2000.

Grant

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NIH/NCI
Genetic Epidemiology of Prostate Cancer Aggressiveness
This new component of CaP Genes is undertaking an association study of candidate genes and regions in advanced prostate cancer, investigating whether the genetic basis of this disease is modified by ethnicity.

Conclusions

During the current reporting period of this Idea Development Award we have successfully used an innovative, multi-faceted association, linkage, and LOH approach to search for genes involved with the occurrence and aggressiveness of prostate cancer. The accomplishments outlined above provide a substantial contribution to the understanding of genetic mechanisms involved with prostate cancer development and aggressiveness, and thus to the programmatic goal of conquering prostate cancer.

One might ask "So What?" about our research findings. Information from our work may provide new molecular markers to help improve screening and treatment for this disease. The ultimate value of this project's successes will reflect our ability to use the resulting information to predict which individuals may be more susceptible to developing prostate cancer, and among those with the disease, to foster therapeutic strategies that adequately reflect risk of progression and recurrence

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Paris PL, Witte JS, Kupelian PA, Levin H, Klein EA, Catalona WJ, Casey G. Identification and fine mapping of a region showing a high frequency of allelic imbalance on chromosome 16q23.2 that corresponds to a prostate cancer susceptibility locus. Cancer Research 2000;60:3645-3649.

Suarez BK, Lin J, Burmester JK, Broman K, Weber JL, Banerjee TK, Goddard KA, Witte JS, Elston RC, Catalona WJ. A genome screen of multiplex sibships with prostate cancer. American Journal of Human Genetics 2000a;66:933-944.

Suarez BK, Lin J, Witte JS, Conti DV, Resnick MI, Klein EA, Burmester JK, Vaske DA, Banerjee TK, Catalona WJ. Replication linkage study for prostate cancer susceptibility genes. Prostate 2000b;45:106-114.

Witte JS, Goddard KAB, Conti DV, Elston RC, Lin J, Suarez BK, Broman KW, Burmester JK, Weber JL, Catalona WJ. Genome-wide scan for prostate cancer aggressiveness loci. American Journal of Human Genetics 2000;67:92-99.

Appendices

1) Suarez BK, Lin J, Burmester JK, Broman K, Weber JL, Banerjee TK, Goddard KA, Witte JS, Elston RC, Catalona WJ. A genome screen of multiplex sibships with prostate cancer. American Journal of Human Genetics 2000;66:933-944.

2) Suarez BK, Lin J, Witte JS, Conti DV, Resnick MI, Klein EA, Burmester JK, Vaske DA, Banerjee TK, Catalona WJ. Replication linkage study for prostate cancer susceptibility genes. Prostate 2000;45:106-114.
3) Witte JS, Goddard KAB, Conti DV, Elston RC, Lin J, Suarez BK, Broman KW, Burmester JK, Weber JL, Catalona WJ. Genome-wide scan for prostate cancer aggressiveness loci. American Journal of Human Genetics 2000;67:92-99.
4) Paris PL, Witte JS, Kupelian PA, Levin H, Klein EA, Catalona WJ, Casey G. Identification and fine mapping of a region showing a high frequency of allelic imbalance on chromosome 16q23.2 that corresponds to a prostate cancer susceptibility locus. Cancer Research 2000;60:3645-3649.

Am. J. Hum. Genet. 66:933–944, 2000

A Genome Screen of Multiplex Sibships with Prostate Cancer

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Analysis of a genome screen of 504 brothers with prostate cancer (CaP) who were from 230 multiplex sibships identified five regions with nominally positive linkage signals, on chromosomes 2q, 12p, 15q, 16p, and 16q. The strongest signal in these data is found on chromosome 16q, between markers D16S515 and D16S3040, a region suspected to contain a tumor-suppressor gene. On the basis of findings from previous genome screens of families with CaP, three preplanned subanalyses were carried out, in the hope of increasing the subgroup homogeneity. Subgroups were formed by dividing the sibships into a group with a positive family history (FH+) that met criteria for "hereditary" CaP ($n = 111$) versus those which did not meet the criteria ($n = 119$) and by dividing the families into those with a mean onset age below the median ($n = 115$) versus those with a mean onset age above the median ($n = 115$). A separate subanalysis was carried out for families with a history of breast cancer (CaB+ [$n = 53$]). Analyses of these subgroups revealed a number of potentially important differences in regions that were nonsignificant when all the families were analyzed together. In particular, the subgroup without a positive family history (FH−) had a signal in a region that is proximal to the putative site of the HPC1 locus on chromosome 1, whereas the late-age-at-onset group had a signal on 4q. The CaB+ subgroup revealed a strong linkage signal at 1p35.1.

Introduction

This year, prostate cancer (CaP) will be the most commonly diagnosed visceral cancer and the second leading cause of cancer mortality among men in the United States (Landis et al. 1999). The prevalence of CaP varies 20- to 30-fold worldwide. The highest frequency is found in African Americans, and the lowest frequency is found in Asian populations (Parkin et al. 1993; Whittemore 1994). Although immigrant (Staszewski and Haenszel 1965; Dunn 1975) and lifestyle and dietary studies (Whittemore et al. 1995*a*) point to the importance of environmental factors, twin (Grönberg et al. 1994; Ahlbom et al. 1997), "kinship" (Cannon et al. 1982; Holloway and Sofaer 1992*a*, 1992*b*), and family studies (Morganti et al. 1956; Woolf 1960; Steele et al. 1971; Krain 1974; Meikle et al. 1985; Steinberg et al. 1990; Ghadirian et al. 1991; Spitz et al. 1991; Keetch et al. 1995; Whittemore et al. 1995*b*) point to the importance of genetic factors. The two strongest predictors of increased risk for CaP, apart from age, are the presence of several affected first-degree relatives and an affected brother who had an unusually early age at onset (Keetch et al. 1995).

Segregation analysis has suggested that some cases of CaP are due to an autosomal susceptibility locus with an allele or alleles that collectively behave in a dominant and age-dependent fashion (Carter et al. 1992; Grönberg et al. 1997*a*; Schaid et al. 1998). Other investigators have argued either for a recessive mode of inheritance or, on the basis of an excess risk of CaP in men with affected brothers compared with men with affected fathers, for an X-linked mode of transmission (Monroe et al. 1995). Unlike breast cancer (Miki et al. 1994; Tavtigian et al. 1996) or colorectal cancer (Fearon et al. 1990; Groden et al. 1991), however, no susceptibility loci with alleles sufficient to cause CaP have yet been identified.

We report here the results of a genome screen of 230 multiplex sibships with CaP.

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Families and Methods

Families

Since 1991, we have been collecting information about multiplex sibships with CaP. No ascertainment criteria, other than the presence of two or more brothers

with documented CaP and a willingness to participate, have been used to recruit the members of the sample population. Approximately half of these subjects were patients of Washington University School of Medicine (WUSM) staff urologists, were referred by other urologists or CaP support groups, or responded to our publications soliciting participation. The remainder were referred by family members enrolled in our studies. The study protocol was approved by the Human Studies Committee of Washington University. Informed consent was obtained from all subjects. All probands and many of their affected brothers completed a family-history questionnaire that was used to partition the sample for various preplanned subanalyses.

A total of 513 subjects were genotyped. Subsequent analyses (see below) reduced this sample to 504 men with CaP. The subjects' mean age at the time of diagnosis was 65.5 years (median, 65.4 years; range, 42–91 years).

The diagnosis of CaP was confirmed directly by WUSM pathologists or by examination of the medical records in 502 (99.6%) of the subjects. Pathologic documentation was missing from two subjects (0.4%); however, in these subjects, the diagnosis of CaP was affirmed by treatment records.

Seventy-six percent of the patients were treated primarily with radical prostatectomy, 10.3% were treated with radiation therapy, 2.6% were treated with primary hormonal therapy, 2.6% were managed with watchful waiting, and 8.5% received miscellaneous other treatments. Of the patients treated primarily with radical prostatectomy, 15.1% have also been treated with hormonal therapy and 7.8% have been treated with radiation therapy.

Genotyping

All samples were genotyped at the Center for Medical Genetics, Marshfield Medical Research Foundation, by use of Weber screening set 9 (Yuan et al. 1997), which consists of simple tandem-repeat polymorphisms, including 366 autosomal, 16 X-linked, and 4 Y-linked markers. Average marker heterozygosity was 77%, and average spacing on sex-equal maps was 9 cM (Broman et al. 1998).

A multipoint linkage analysis (see below) was used to rank the markers according to the estimated mean allele sharing among affected brothers. Regions around the highest-ranking nine markers were selected for further genotyping. An additional 38 microsatellite markers (~4.2/signal) were genotyped at the Center for Medical Genetics, Marshfield Medical Research Foundation. The average spacing between adjacent markers for the 38 new intervals created by this second wave of genotyping was 2.2 cM.

Statistical Methods

Before conducting the linkage analysis, we assessed the marker genotypes to verify the status of each alleged sib pair, using two approaches: the RELATIVE program (Göring and Ott 1995) and a modified version of the RELPAIR program (Boehnke and Cox 1997; Broman and Weber 1998). The results were similar with both programs and revealed the presence of one half-sib (from an affected trio) and four sets of twins (one pair of which was from an affected trio) who, by genotyping, were shown to be MZ. An additional subject was dropped from the affected sample after the genome screen had been completed because a record review indicated that he did not have CaP. After these nine individuals had been deleted from the sample, 504 full sibs from 230 nuclear families remained for linkage analysis. The familial distribution of genotyped brothers used in this analysis was as follows: 188 affected pairs, 40 affected trios, and 2 affected quartets.

To determine if alleles at the microsatellite markers were in Hardy-Weinberg proportions in this sample, we carried out likelihood-ratio tests with the ASSOC program (Ott 1985), choosing, from each family at random, one genotyped sib per marker.

Despite the evidence from three separate segregation analyses—all of which argue that a sizable proportion of CaP cases (particularly early-onset cases) are due to a highly penetrant dominant gene—a susceptibility locus has not yet been identified. Therefore, rather than compute linkage statistics under what may eventually prove to be a grossly inaccurate model, we preferred to compute allele-sharing statistics that do not require specification of the mode of transmission. Since the original implementation of the nonparametric-linkage (NPL) scoring algorithm has been shown to be overly conservative when data on parental genotypes are lacking (Davis and Weeks 1997; Badner et al. 1998), we decided to compute the Kong-and-Cox (KAC) statistic (Z_{lr} score) as implemented in GENEHUNTER-PLUS (Kruglyak et al. 1996; Kong and Cox 1997), using the exponential model option, the "pairs" scoring function, and equal weights for each family. A related program, MAPMAKER/SIBS (version 2.0; Kruglyak and Lander 1995), was used to estimate the mean proportion of alleles shared identical by descent. Allele frequencies were estimated from the data, and for all subanalyses, allele frequencies were re-estimated for each data partition examined.

Under the null hypothesis, Z_{lr} scores have a standard normal distribution. When the ith Z_{lr} score from one data partition is compared with its complementary partition, the statistic

$$D = \frac{|Z_{lr_C} - Z_{lr_{(1-C)}}|}{[2(\text{var } Z_{lr}) - \text{cov } Z_{lr}]^{1/2}}$$

is asymptotically $N(0,1)$. "C" and "$1 - C$" denote the two data partitions. Since all subanalyses partition the data by family units, and since the families are independent, the covariance term in the above expression is 0. With one notable exception (a predicted increase in the Z_{lr} score of the early-age-at-onset partition for some chromosome 1 regions), we have no prior hypotheses about the direction of any differences that could result from partitioning of the data. Accordingly, for the sake of conservative consistency, all tests of the significance of D are two tailed.

Results

Multipoint Z_{lr} scores for all chromosomes (except the Y chromosome) are displayed in figure 1. Five chromosomal regions gave nominal evidence for linkage (i.e., a Z_{lr} score >1.645) at two or more adjacent markers: (1) a very broad region on 2q, extending ~66 cM, from D2S1391 to D2S2968; (2) a narrow region on 12p, extending ~3.03 cM, from D12S1615 to D12S1685; (3) a moderately sized region on 15q, extending ~19.2 cM, from D15S822 to the dinucleotide repeat in the actin alpha cardiac-muscle gene; (4) a broad region on 16p, extending ~39.1 cM, from ATA41E04 to the centromere; and (5) a moderate region on 16q, extending ~16.8 cM, from D16S2624 to D16S3040. Table 1 reports the marker at which the maximum Z_{lr} score occurs, for each of the aforementioned five chromosomal regions as well as the estimate of the mean proportion of alleles shared identical by descent. Only three markers gave nominal (i.e., $P < .05$) evidence of departure from Hardy-Weinberg equilibrium (GATA91H06, $P = .006$; ATA78D02, $P = .047$; and D18S970, $P = .049$), and none are located in any of the above five regions.

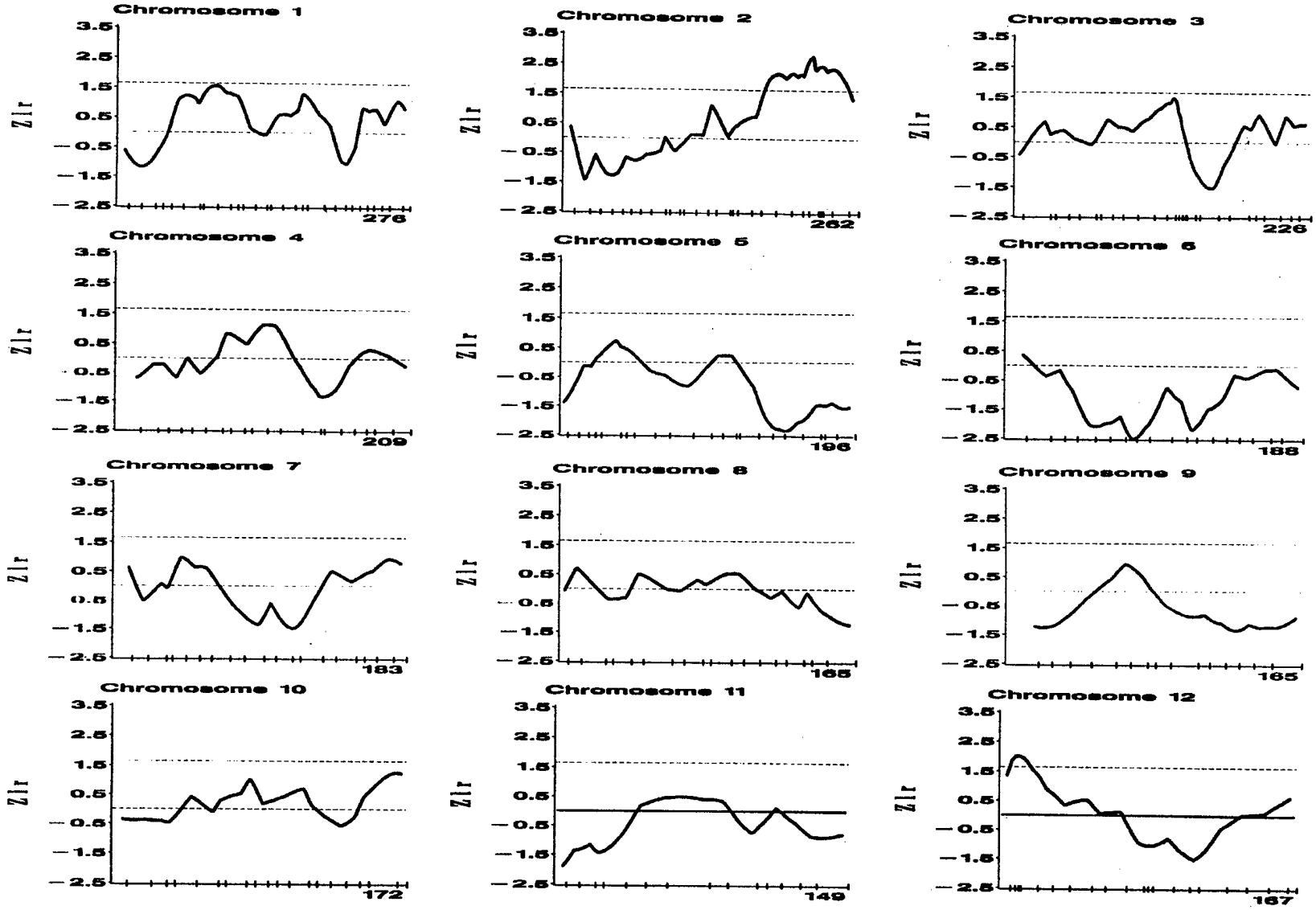
We preplanned three subanalyses that involved dichotomizing the total sample into subsets that previous research has suggested may increase homogeneity in the subgroups. χ^2 Analyses indicate that the various family partitions are not significantly pairwise-dependent in these data (table 2). As noted above, allele frequencies were re-estimated whenever a new partition of the families was constructed. Since chromosome 16 yielded moderate-to-strong signals for all family partitions, the Z_{lr} scores for this chromosome are presented separately.

It is unclear how best to report the results of a whole-genome screen when various subgroups are analyzed separately. The fact that these subgroup analyses were preplanned does not mean that a price need not be paid for performance of multiple tests. On the other hand, for a complex and heterogeneous phenotype, such as CaP, complete genetic characterization—including precise specification of all gene-gene and gene-environment interactions—may require sample sizes that are an order of magnitude larger than currently available. We have chosen to report all adjacent markers (i.e., two or more) for which the Z_{lr} score is nominally significant at $P < .05$. Since these P values are uncorrected for multiple tests, most will prove to be false positives.

The first subanalysis involved partitioning the families according to whether they met the "Hopkins" criteria for "hereditary" CaP due to features of their family history (FH). To be classified as belonging to the FH-positive (FH+) group, a family must contain either (1) two or more brothers with a diagnosis of CaP at age ≤ 55 years, (2) at least three first-degree relatives with a diagnosis of CaP, or (3) three consecutive generations with CaP (Carter et al. 1992). Since our study design required, at a minimum, the presence of at least one affected sib pair (ASP), any family meeting criterion 3 would necessarily also meet criterion 2. Only six of the families under study met criterion 1, and five of them also had an affected father. Consequently, virtually all of our FH+ families were so classified because they met criterion 2. One hundred eleven families containing a total of 199 ASPs met the criteria for FH+. Table 3 reports the distribution of nominally significant Z_{lr} scores achieved in either partition. A number of interesting contrasts are apparent.

Although no nominally significant evidence of linkage of chromosomes 1, 3, 8, or 18 was obtained when all of the families were analyzed together, the FH+/FH− partitioning reveals such evidence. With respect to the signals on chromosome 1, the two regions are separated by ~57.8 cM, so, despite their synteny, they are unlinked. Accordingly, it is noteworthy that, for the two blocks of adjacent chromosome 1 markers, when one group or the other attained a nominally significant Z_{lr} score, the partitioning resulted in a significant difference between the two subgroups. Of the 33 non–chromosome 1 markers listed in table 3, only one, on chromosome 8 (GAAT1A4), reveals a significant between-subgroup difference.

A consistent finding in cancer genetics (Giardiello 1997) is that families with an early age at onset appear to have higher "genetic loading" and, in some cases, a single major locus with alleles sufficient to cause the cancer. Therefore, we subdivided our sample according to each family's mean age at onset. The means were ranked, and a median split resulted in 115 families in each subgroup. Nominally significant Z_{lr} scores for this partition are reported in table 4. Interestingly, with the exception of two contiguous markers on chromosome 12 and two contiguous markers on chromosome 15, the highest Z_{lr} scores are observed in the families with the latest mean age at onset. Of the 35 markers listed in


Chromosome 1
Chromosome 2
Chromosome 3
Chromosome 4
Chromosome 5
Chromosome 6
Chromosome 7
Chromosome 8
Chromosome 9
Chromosome 10
Chromosome 11
Chromosome 12
Zlr
3.5
2.5
1.5
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276
262
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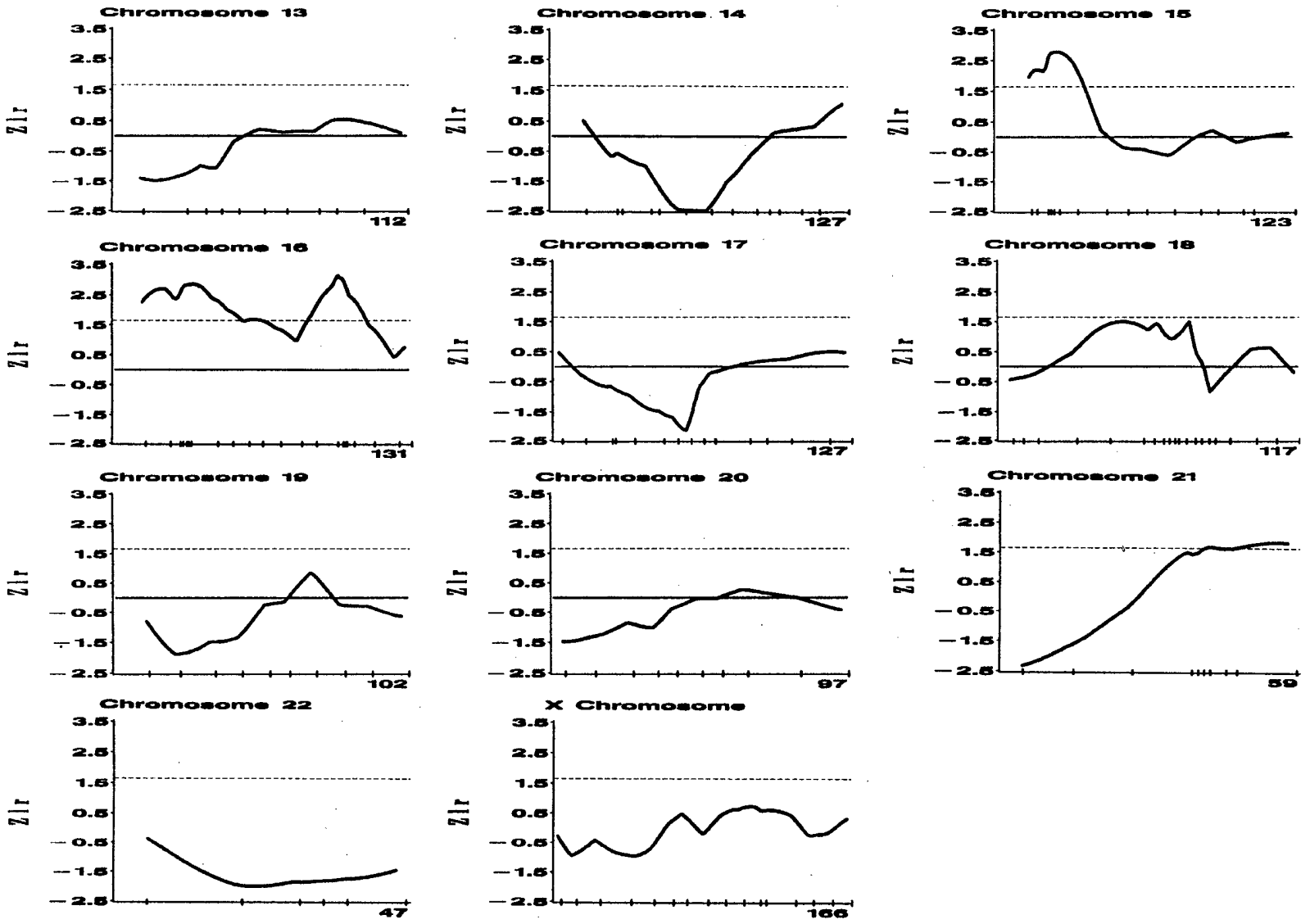

Figure 1 Z_{lr} scores for 420 markers genotyped on 504 brothers with CaP who were from 230 multiplex sibships. The check marks show the positions of the markers. The total length of each chromosome (in cM) is shown in the lower-right corner.

table 4, the median split resulted in 13 (37%) significant between-group differences in their Z_{lr} scores. And in all but 2 of these 13 significant differences, the linkage signal occurs in the late-age-at-onset group.

On the basis of recent observations by Cerhan et al. (1999), we partitioned the families according to whether they were positive for breast cancer (CaB+). To qualify for classification within the CaB+ group, the proband had to report the presence of breast cancer in a sister, mother, biological aunt, or grandmother. These self-reports were not verified. Fifty-three of the 230 multiplex families contained one or more cases of breast cancer (no cases of male breast cancer were reported). These 53 families contained a total of 81 ASPs. Five adjacent markers on 1p and seven adjacent markers on 21q in the CaB+ partition yielded nominally significant Z_{lr} scores (table 5).

The most consistent finding from our genome screen is the suggestion of susceptibility loci on chromosome 16. Table 6 reports the Z_{lr} scores for all 22 markers typed on chromosome 16, both for the entire sample and for the various partitions. It is perhaps noteworthy that, whereas the various subgroups reported in tables 3–5 suggest a degree of chromosomal specificity, little is evident for chromosome 16. Only for families in the late-age-at-onset partition (and only for 16q markers) is there an absence of even a nominally significant signal. With the exception of markers D16S539 and D16S2621 in the early- versus late-age-at-onset comparison, none of the data partitions resulted in a significant difference between any of the subgroups.

Table 1

Allele Sharing in ASPs, for Five Chromosomal Regions That Yield Nominal Evidence for Linkage

Chromosome	Marker	Posiiton (cM)	Z_{lr}	Mean ASP Allele Sharing
2q	D2S2228	224.33	2.78	.557
12p	D12S1685	7.67	2.00	.533
15q	D15S1010	23.89	2.77	.544
16p	D16S3103	32.07	2.81	.546
16q	D16S3096	99.44	3.15	.563

Discussion

Susceptibility loci that predispose to diseases with a late mean age at onset are notoriously difficult to map. The proband's parents are usually deceased, and, even if DNA were available on all members of the sibship, the sibship may be too small to allow unambiguous reconstruction of the parental genotypes. Depending on the particulars of the disease, the proband's offspring are unlikely to be old enough to be informative. These difficulties certainly apply to CaP, in which the sex-limited nature of the disease further reduces the available information. These factors help to explain why no undisputed susceptibility locus has yet been identified for CaP and why it is proving so difficult to achieve unambiguous replication in linkage studies.

We report here the results of a linkage study of 230 multiplex sibships with CaP, using a total of 420 highly polymorphic markers. Although five different chromosomal regions gave nominal evidence of a possible susceptibility locus, none of the signals in the total sample is sufficiently strong to meet the Lander and Kruglyak's (1995) threshold for "suggestive" linkage (i.e., a $P = .00074$ or $Z_{lr} \approx 3.18$). And, although the usefulness of this criterion has been questioned (Curtis 1996; Witte et al. 1996), it is clear that oligogenic phenotypes—even those with 100% heritability—may result in increased sib-pair allele sharing that is only a few percentage points over the null value of 50% (Suarez et al. 1994).

Linkage studies have been successful in the identification of disease-susceptibility loci, including many that predispose to cancer (Fearon et al. 1990; Hall et al. 1990; Groden et al. 1991; Miki et al. 1994; Tavtigian et al. 1996). However, there are surprisingly few linkage studies of CaP. The first and only complete genome screen published to date was reported by Smith et al. (1996) and presented evidence of a susceptibility locus (HPC1 [MIM 601518]) on the long arm of chromosome 1 at 1q24-q25. A subsequent reanalysis of an expanded collection of the multiplex pedigrees in their study suggested that families with an early age at onset were primarily responsible for the linkage signal at HPC1 (Grönberg et al. 1997*b*, 1999). This claim remains controversial. Two other studies have produced modest support for the existence of HPC1. Cooney et al. (1997)

Table 2

Distribution of Families Cross-Classified According to Criteria Used to Partition the Data for Preplanned Subanalyses

	Early Age at Onset				Late Age at Onset			
	FH+		FH−		FH+		FH−	
	CaB−	CaB−	CaB−	CaB−	CaB+	CaB−	CaB+	CaB−
No. of families	16	41	14	44	10	44	13	48
No. of ASPs	31	67	14	44	23	78	13	48

Table 3

Nominally Significant KAC Z_{lr} Scores and *P* Values for Families with CaP, Subdivided According to Whether They Are FH+ or FH−

Marker[a]	Position (cM)	KAC Z_{lr} Score (*P*) in[b] FH+ Families	FH− Families
D1S534	151.88		1.65 (.050)
D1S1653	164.09	...*	1.86 (.031)
D1S1679	170.84	...**	2.28 (.011)
D1S1677	175.62	...**	2.72 (.003)
D1S2141	233.38	2.10 (.018)	...*
D1S549	239.66	1.84 (.034)	...*
D2S1384	200.43	2.02 (.022)	
D2S2944	210.43	2.23 (.012)	
D2S434	215.78	2.15 (.016)	
D2S2228	224.33	2.22 (.013)	
D2S2390	225.67	1.76 (.039)	
D2S1363	227.00	1.85 (.032)	
D2S159	228.61	1.94 (.026)	
D2S427	236.70	2.25 (.012)	
D2S2968	251.94	2.25 (.012)	
D2S125	260.63	1.72 (.042)	
D3S4529	112.42		1.78 (.038)
D3S2459	119.09		2.15 (.016)
D3S1591	121.67		2.01 (.022)
D3S3045	124.16		1.94 (.026)
D3S1616	124.16		1.94 (.026)
D3S3695	124.83		1.73 (.042)
D8S1119	101.01	1.67 (.047)	
GAAT1A4	110.20	2.36 (.009)	...***
D15S822	12.30		1.80 (.036)
D15S1002	14.58		1.92 (.027)
D15S1048	19.12	1.89 (.029)	
D15S165	20.24	1.65 (.050)	
D15S184	21.58	1.83 (.033)	2.01 (.022)
D15S1010	23.89		2.34 (.009)
ACTC	31.46		2.74 (.003)
GATA173A03	54.40		1.87 (.031)
D18S535	64.48		1.70 (.045)
GATA81H03	66.66		1.80 (.036)
D18S970	68.30		1.94 (.026)
D18S363	71.32		2.09 (.018)
D18S851	74.93		1.95 (.025)
D18S539	74.93		1.95 (.025)
ATA82B02	106.81		1.75 (.040)

[a] Chromosome 16 markers are not included.

[b] Asterisks denote level of significance between the respective Z_{lr} scores of each subgroup: * = $.05 > P > .01$, ** = $.01 > P > .001$, and *** = $P < .001$.

reported an analysis of 59 multiplex families and obtained an NPL Z-score of 1.58 ($P = .057$) at D1S466. An analysis of the 20 families that met criteria for "hereditary" CaP produced an NPL Z-score of 1.72 ($P = .045$) at D1S466. Hsieh et al. (1997) obtained equivocal results in a sample of 92 multiplex families. When these families were subdivided according to the family's mean age at onset, a nominally significant signal in the younger group was detected at D1S452 (two-point $Z = 2.04$, $P = .023$), and another modest signal was detected at D1S2883 (two-point $Z = 1.91$, $P = .030$), in the late-age-at-onset partition. Since these two markers are only about 5.5 cM apart, these results suggest that there could be two different CaP-susceptibility loci on 1q.

Three of the markers we typed map within the putative HPC1 region, and none approach nominal significance in the total sample. However, nominally significant linkage is obtained for a block of four proximal markers in the FH− partition and for two distal markers in the FH+ subgroup. Since our FH+ signal occurs approximately 20 cM from the closest HPC1 marker, this should not be interpreted as a replication. Three other studies have been unable to confirm the existence

Table 4

Nominally Significant KAC Z_{lr} Scores and *P* Values Families with CaP, Subdivided by Median Age at Onset

Marker	Position (cM)	KAC Z_{lr} Score (*P*) in Families with Mean Age at Onset in Lower 50th Percentile	Families with Mean Age at Onset in Upper 50th Percentile
D1S547	267.51	...*	2.06 (.020)
D1S1609	274.53	...*	2.01 (.022)
D2S2944	210.43		1.99 (.024)
D2S434	215.78		2.16 (.016)
D2S2228	224.33		2.92 (.002)
D2S2390	225.67		2.57 (.005)
D2S1363	227.00		2.35 (.009)
D2S159	228.61		2.36 (.009)
D2S427	236.70		2.16 (.015)
D2S2968	251.94		1.67 (.048)
D4S2367	78.43	...*	2.28 (.011)
D4S3243	88.35	...*	1.85 (.032)
D4S1647	104.94	...**	2.72 (.003)
D4S2623	114.04	...**	2.85 (.002)
D4S2394	129.92	...**	1.92 (.027)
ATA34E08	33.02	...***	1.87 (.031)
D11S1392	43.16	...**	2.17 (.015)
D12S1685	7.67	1.85 (.032)	
GATA49D12	17.72	1.80 (.036)	
D12S2070	125.31	...**	1.65 (.050)
D12S395	136.82	...**	1.98 (.024)
D15S1002	14.58		1.65 (.049)
D15S1048	19.12		2.10 (.018)
D15S165	20.24		2.37 (.009)
D15S184	21.58		2.50 (.006)
D15S1010	23.89		3.01 (.001)
ACTC	31.46		3.00 (.001)
D15S657	104.86	1.71 (.043)	...*
D15S642	122.14	1.68 (.047)	...*
D21S1440	36.77		2.22 (.013)
D21S270	38.08		2.05 (.020)
D21S1255	39.22		2.06 (.020)
D21S2055	40.49		2.08 (.019)
D21S1893	43.6		2.15 (.016)
D21S266	45.8		2.12 (.017)

NOTE.—See footnotes to table 3.

Table 5

Nominally Significant Scores for Families with CaP That Are CaB+

Marker	Position (cM)	Z_{lr} (*P*)
D1S552	45.33	1.89 (.029)
D1S1622	56.74	3.78 (<.001)
D1S3721	72.59	2.20 (.014)
D1S2134	75.66	1.87 (.030)
D1S3728	89.49	2.37 (.009)
D21S1440	36.77	2.68 (.003)
D21S270	38.08	2.86 (.002)
D21S1255	39.22	2.83 (.002)
D21S2055	40.49	2.90 (.002)
D21S1893	43.67	2.36 (.009)
D21S266	45.87	2.21 (.013)
D21S1446	57.77	2.03 (.021)

NOTE.—See footnote "a" to table 3.

of HPC1 (McIndoe et al. 1997; Berthon et al. 1998; Eeles et al. 1998).

Two other regions of chromosome 1 have also been reported to harbor CaP-susceptibility loci. Berthon et al. (1998) reported an NPL *Z* score of 3.1 ($P < .001$) in the vicinity of 1q42.2-q43 in 47 French and German families. The NPL *Z* score increased to 3.32 in a subset of nine families in which the mean age at onset was <60 years. Homogeneity analysis led Berthon et al. (1998) to estimate that this putative susceptibility locus (PCAP; MIM 602759) accounts for <50% of the "hereditary" CaP cases in their data. Only two markers from the Weber 9 set map within this region, and, in our total sample, the Z_{lr} score is <1.0, for both markers. In our subgroup analyses, we obtained nominally significant evidence of linkage for these two markers—but, in the families that we studied, the signal comes from the late-age-at-onset partition. Recently, Gibbs et al. (1999*a*) reported negative LOD scores for four markers from this distal region of chromosome 1q, and Whittemore et al. (1999) reported negative NPL *Z*-scores for the same four markers.

In a separate report, Gibbs et al. (1999*b*) presented evidence for a rare susceptibility locus, at 1p36, that appears to be important only in families that also have primary brain cancer. Although we did not preplan to analyze our families according to the presence of brain cancer, we conducted such an analysis of just chromosome 1p, once the report by Gibbs et al. (1999*b*) appeared. Only 13 families in our sample have a history of brain cancer, so we have little power to confirm the linkage. Three of the markers that we genotyped are located in the vicinity of the signal reported by Gibbs et al. (1999*b*), and, for all three markers, nonsignificant positive Z_{lr} scores were obtained (Z_{lr} = 0.98, 1.15, and 1.49 at D1S1597, D1S3669, and D1S552, respectively).

Recently, Xu et al. (1998) presented evidence of an X-linked susceptibility locus (HPCX [MIM 300147]), at Xq27-q28, that, they estimate, accounts for ~16% of "hereditary" cases of CaP. In this region, the only X-linked marker genotyped in the sibships that we studied was GATA31E08, at Xq27.1. For the entire sample, we obtained a multipoint Z_{lr} score of −0.163, and none of the various data partitions produced a Z_{lr} score >0.81.

The strongest linkage signal in our genome screen of the entire sample occurred on the long arm of chromosome 16, at 16q23.2. Analysis of the various subsamples indicated that no family partition disproportionately accounts for these signals. A maximum Z_{lr} score of 3.15 is obtained at D16S3096.

Loss of heterozygosity (LOH) studies in CaP tumors have consistently found an increased loss on chromosome 16q (as well as 8p and 10q [Carter et al. 1990; Bergerheim et al. 1991; Cher et al. 1995; Elo et al. 1997; Osman et al. 1997]). Indeed, the pattern and distribution of LOH on 16q has led to speculation that up to three distinct susceptibility loci important for tumorigenesis, metastasis, or both may be present (Suzuki et al. 1996; Latil et al. 1997). One of these regions is located in the vicinity of our strongest signal. All previous studies of LOH in CaP tumors have been carried out in unrelated individuals. If the moderate signal that we have observed in these data is not a type I error, then it raises the possibility that a proportion of the families in our sample may be segregating an allele at a tumor-suppressor gene in this region; and, according to the Knudson (1971) model, all that is required to initiate tumorigenesis is a second somatic mutation in a single prostate cell.

Although we were able to verify the diagnosis of CaP by histological means or medical-record review in all but two of our subjects (and those two received treatment consistent with the diagnosis), the information regarding a family history positive for breast cancer was obtained from the probands, and no attempt to verify it was made. Two genomic regions—a broad region containing five markers and covering ~45 cM on chromosome 1p and a 21-cM region on chromosome 21q—yielded nominally significant Z_{lr} scores. The Z_{lr} score at D1S1622 (3.78) corresponds to a LOD score >3 and meets criteria for suggestive linkage.

The short arm of chromosome 1 frequently shows allelic loss in breast cancer tumors (Schwab et al. 1996; Bieche et al. 1999; Perri et al. 1999). The c-myc promoter–binding protein, MPB1, which suppresses tumorigenicity in breast cancer cells, has been mapped to the p35-pter region of chromosome 1 (White et al. 1997). In a recent study, Millikan et al. (1999) report frequent LOH at two 1p36 markers (D1S243 and D1S160), but no evidence of linkage was obtained from an analysis of families with a history of early-onset bi-

Table 6

Chromosome 16 Multipoint KAC Z_{lr} Scores for the Total Sample and for the Three Data Partitions

		KAC Z_{lr} Score in					
Marker	Position (cM)	All Families	FH+ Families	FH− Families	Families with Early Age at Onset	Families with Late Age at Onset	CaB+ Families
ATA41E04	11.46	2.17	2.07	1.08	1.40	1.71	2.48
D16S748	22.65	2.71	1.93	1.94	1.64	2.20	2.14
D16S3062	27.05	2.34	1.65	1.71	1.16	2.16	1.99
D16S405	28.30	2.38	1.60	1.78	1.31	2.08	1.87
D16S764	29.97	2.61	1.54	2.18	1.66	2.05	1.92
ATA63G01	30.81	2.80	1.66	2.34	1.89	2.12	1.93
D16S3103	32.07	2.81	1.74	2.28	1.96	2.07	1.69
D16S403	43.89	2.37	1.43	1.86	2.08	1.20	1.10
D16S769	50.60	1.99	1.88	.90	1.70	1.05	.62
Centromere							
D16S753	57.79	1.61	2.14	.16	1.16	1.04	−.37
D16S3396	63.78	1.67	2.46	−.04	1.28	1.12	−.46
D16S3253	71.77	1.40	2.43	−.38	1.26	.75	−.12
GATA67G11	81.15	.95	1.92	−.64	1.25	−.01	.16
D16S2624	87.62	1.81	2.30	.12	2.11	.36	.93
D16S3049	97.03	2.80	2.00	1.84	2.27	1.57	1.60
D16S3096	99.44	3.15	2.06	2.30	2.82	1.56	2.08
D16S516	100.39	3.07	2.12	2.14	2.83	1.46	1.95
D16S504	101.23	3.08	2.13	2.12	2.86	1.42	2.02
D16S3040	104.45	2.48	2.23	1.19	2.62	.77	1.62
D16S402	113.52	1.48	1.44	.49	2.25	−.34	1.35
D16S539	124.73	.41	.90	−.48	1.97	−1.53	.53
D16S2621	130.41	.82	.80	.18	2.08	−1.04	.84

lateral breast cancer. Our results in the CaB+ partition raise the possibility that one or more tumor-suppressor genes capable of inhibiting tumorigenesis in both breast and prostate cells may be located on the short arm of chromosome 1.

As is the case with most complex diseases, polymorphisms in a number of candidate genes have been proposed as increasing the risk for CaP. Alleles at these loci are not believed to be necessary or sufficient to cause CaP, any more than the Apo ε4 allele is sufficient to cause Alzheimer disease; rather, they are risk factors in the epidemiological sense. Among these loci are the steroid 5-alpha-reductase 2 gene (Reichardt et al. 1995), on 2p23; the vitamin D–receptor gene (Taylor et al. 1996; Ingles et al. 1998), on 12q12-q14; the homeobox 3A gene (Abbaszadegan et al. 1998), on 8p21; and the X-linked androgen-receptor (AR) gene, on Xq11-q12, which contains in its first exon two polymorphic trinucleotide repeats—a 5′ CAG repeat and a 3′ GGC repeat. Given the central role played by androgens in the development and maintenance of normal prostate, and given that the length of the CAG repeat is inversely correlated with transcriptional activity, it is not surprising that these AR polymorphisms have received a great deal of attention. Hardy et al. (1996) found a significant correlation between the CAG-repeat number and an early age at onset of CaP, whereas Giovannucci et al. (1997) found that men with shorter repeats were at particularly high risk for distant metastatic and fatal CaP. A recent case-control study in a French and German sample, however, found no association between these polymorphisms and risk for CaP (Correa-Cerro et al. 1999). Although we did not type any of these candidate genes, our genome screen revealed no signals in the regions where these candidates map.

Prior to conducting any of the linkage analyses, we preplanned to partition our sample according to variables that reasonably might produce greater homogeneity in the subgroups. Two of these partitions were based on FH: families that met the Hopkins criteria for hereditary CaP were compared with families that may be sporadically multiplex. The second subanalysis focused on sibships from families that were CaB+. The third partitioning used age at onset to divide the families into two equal groups according to whether the sibship's mean age at onset was below or above the sample's median. The use of a median split in the present study is entirely arbitrary, since age at onset in our sample does not deviate from normality (Shapiro-and-Wilk [1965] test; $W = .988$, $P = .82$). For a number of well-known diseases, including various cancers, either strong FH+ (usually with a dominant-type transmission pattern) or an unusually early age at onset suggests a single segregating susceptibility gene with high penetrance. And, indeed, this association has been exploited successfully to map, clone, and characterize a number of

large-effect susceptibility loci (e.g., BRCA1 and BRCA2) in stringently ascertained pedigrees; however, it is unlikely that genes such as BRCA1 or BRCA2 would be identified in a simple sib-pair study, just as it is unlikely that any of the highly penetrant genes that give rise to Alzheimer disease (i.e., amyloid beta A4–precursor protein, presenilin 1, or presenilin 2) would be identified in a random sample of affected sibs. These major genes are simply too rare. On the other hand, a genome screen of a random sample of sib pairs concordant for Alzheimer disease can detect the linkage signal in the vicinity of the Apo E locus on chromosome 19q, as recently demonstrated by Kehoe et al. (1999).

In the two analyses that compared linkage signals from complementary data partitions (tables 3 and 4), additional nominally significant signals were detected in the partitions—namely, the FH− and the late-age-at-onset partitions—that, on a priori grounds, might be expected to yield a larger proportion of sporadic cases. This excess could be a measure of the increased type I–error rate occasioned by the smaller sample sizes that result from subdivision. Alternatively, some of these signals may reflect the presence of true susceptibility loci that exert an effect, for instance, later in life. Further work will be required to eliminate the false-positive signals.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshfield.org/genetics (for markers and their positions [in cM])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih/gov.Omim (for HPCX [MIM 300147], HPC1 [MIM 601518], and PCAP [MIM 602759])

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Replication Linkage Study for Prostate Cancer Susceptibility Genes

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BACKGROUND. Since the publication of the first genome screen for prostate cancer (CaP) 5 years ago, over a dozen linkage studies have appeared. Most attention has been directed to chromosome 1, where two separate regions have been identified as harboring a prostate cancer susceptibility locus: HPC1 in the 1q24–25 interval and PCaP in the 1q42.2–43 interval. Linkage analysis of chromosome 16 has also provided evidence of harboring two loci predisposing to CaP.

METHODS. We report on a replication linkage study of chromosomes 1 and 16 in 45 new and 4 expanded multiplex CaP families. Multipoint Z-scores were obtained for 30 highly polymorphic short-sequence tandem repeat markers spanning chromosome 1, and 22 markers spanning chromosome 16.

RESULTS. The replication sample gave no evidence for a CaP susceptibility locus in the 1q24–25 interval and equivocal evidence for such a locus at 1q42.2–43. With respect to chromosome 16, positive Z-scores were obtained over a contiguous interval covering the entire p arm and the proximal half of the q arm.

CONCLUSIONS. The linkage analysis of our replication sample does not support the existence of HPC1, and the evidence for the existence of PCaP remains equivocal. Evidence of a

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susceptibility locus on 16p remains strong, but the evidence for a susceptibility locus on 16q is weakened. *Prostate 45:106–114, 2000.* © 2000 Wiley-Liss, Inc.

KEY WORDS: **prostate cancer; linkage analysis; chromosome 1; chromosome 16; replication**

INTRODUCTION

Prostate cancer (CaP) is the most commonly diagnosed visceral cancer and the second leading cause of cancer death among American men [1,2]. With continuing increases in longevity and the aging of the baby-boomer generation, morbidity and mortality due to CaP is expected to consume an increased proportion of the nation's health care resources.

Within the past 5 years there has been a concerted effort to identify specific genes that increase susceptibility to prostate cancer. This search is bolstered, in part, by three independent segregation analyses [3–5] that presented evidence for the existence of at least one major autosomal-dominant gene in the etiology of familial prostate cancer. However, CaP's late age-of-onset makes the task of identifying susceptibility loci difficult, since the parents of affected men are often deceased and their sons may not be through a sufficient portion of the risk period to be informative. Despite these difficulties, two autosomal regions have been identified as likely containing a locus with alleles that predispose to CaP; both are on the long arm of chromosome 1, i.e., HPC1 (MIM 601518) in the 1q24–25 interval [6], and PCaP (MIM 602759) in the 1q42.2–43 interval [7]. In addition, we recently reported that chromosome 16 may also contain two susceptibility loci [8]. Since the existence of HPC1 was first inferred by Smith et al. [6], more than a dozen linkage analyses of chromosome 1 have appeared, but the susceptibility locus inferred from segregation analyses has not yet been identified.

Replication of linkage claims is a critical prerequisite for the eventual identification of a susceptibility gene's coding sequence. We report here on a replication linkage study of 45 new and 4 expanded multiplex families for chromosomes 1 and 16.

METHODS

Families were ascertained from three sites: Washington University Medical School (WUMS), University Hospitals of Cleveland and Cleveland Clinic Foundation (UH/CCF), and Marshfield Medical Research Foundation (MMRF). The only ascertainment criterion used to recruit families was the presence of two or more documented cases of prostate cancer. The research protocols were approved by the Institutional Review Board at each site, and written informed consent was obtained from all participants.

The diagnosis of prostate cancer was confirmed directly by participating pathologists or through examination of medical records. Determination of age-of-onset (i.e., age-at-diagnosis) was made from the examination of medical records or from family history questionnaires completed by the participants.

Tumors were graded using the Gleason system [9]. Gleason grades were recorded from both biopsy specimens and radical prostatectomy specimens. We used the Gleason grade from the latter when available; otherwise, we used the biopsy Gleason grade.

All samples were genotyped for simple tandem-repeat polymorphisms at MMRF, as previously described [10].

Prior to undertaking the linkage analysis, the alleged genetic relationship of the various relative configurations was verified using the computer programs RELATIVE [11] and RELPAIR [12]. GENEHUNTER-PLUS [13,14] was used to compute the Kong-and-Cox linkage statistics (Z-scores) under the exponential model option and the "pairs" scoring function. Equal weights were assigned to each family. Marker allele frequencies were reestimated from the data for each separate linkage analysis to protect against an unintentional increase in type I error that may accompany gene frequency misspecification.

RESULTS

Table I shows the number of families contributed by each of the three sites. Four of the 31 families from WUMS were included in our previous report [8] as 4 independent affected sib-pairs. An additional brother in each family subsequently developed prostate cancer, and these families are included here as affected trios. The replication sample consists of 49 families (all of European ancestry), containing a total of 113 genotyped individuals (97 affected with CaP and 16 either unaffected or female).

To guard against the possibility that there might be unrecognized between-site heterogeneity, an analysis of variance for two important and easily quantifiable parameters of prostate cancer was performed. No between-site difference for either age-of-onset ($F = 1.96$, $P = 0.148$) or Gleason grade ($F = 1.03$, $P = 0.361$) was detected.

TABLE I. Origin of Affected Families by Site*

	Original WUMS families[a]	Replication sample		
		WUMS	UH/CCF	MMRF
Sibs	188	23	7	3
Trios	40	1		2
Quartets	2			1
Half-sibs		3		
First cousins			1	3
Uncle-nephew				1
Sib → Trio[b]		4		
Total	230		49	

*WUMS, Washington University Medical School; UH/CCF, University Hospitals of Cleveland and Cleveland Clinic Foundation; MMRF, Marshfield Medical Research Foundation
[a]See Suarez et al. [8].
[b]A third affected sib was added to an original WUMS affected sib-pair, making it an affected trio.

Two separate linkage analyses on the replication sample and the combined sample were conducted for chromosome 1 and for chromosome 16. For comparison, the results of the new analyses are plotted along with the results from our original genome scan. Figure 1 displays the multipoint Z-scores for the replication sample (N = 49 families) and the combined sample (N = 275 families).

The genome scan of our original 230 families yielded positive, albeit nonsignificant, Z-scores for the three markers we genotyped in the 1q24–25 interval (D1S1679, D1S1677, and D1S1589). For these three markers the replication sample yielded negative Z-scores, though owing to the larger size of the original sample, the combined Z-scores remained positive.

For the 1q42.2–43 region, our original sample gave nonsignificantly positive Z-scores at D1S3462 and D1S235. Figure 1 shows that the replication sample also yielded positive Z-scores for these markers. When the replication sample is pooled with the original

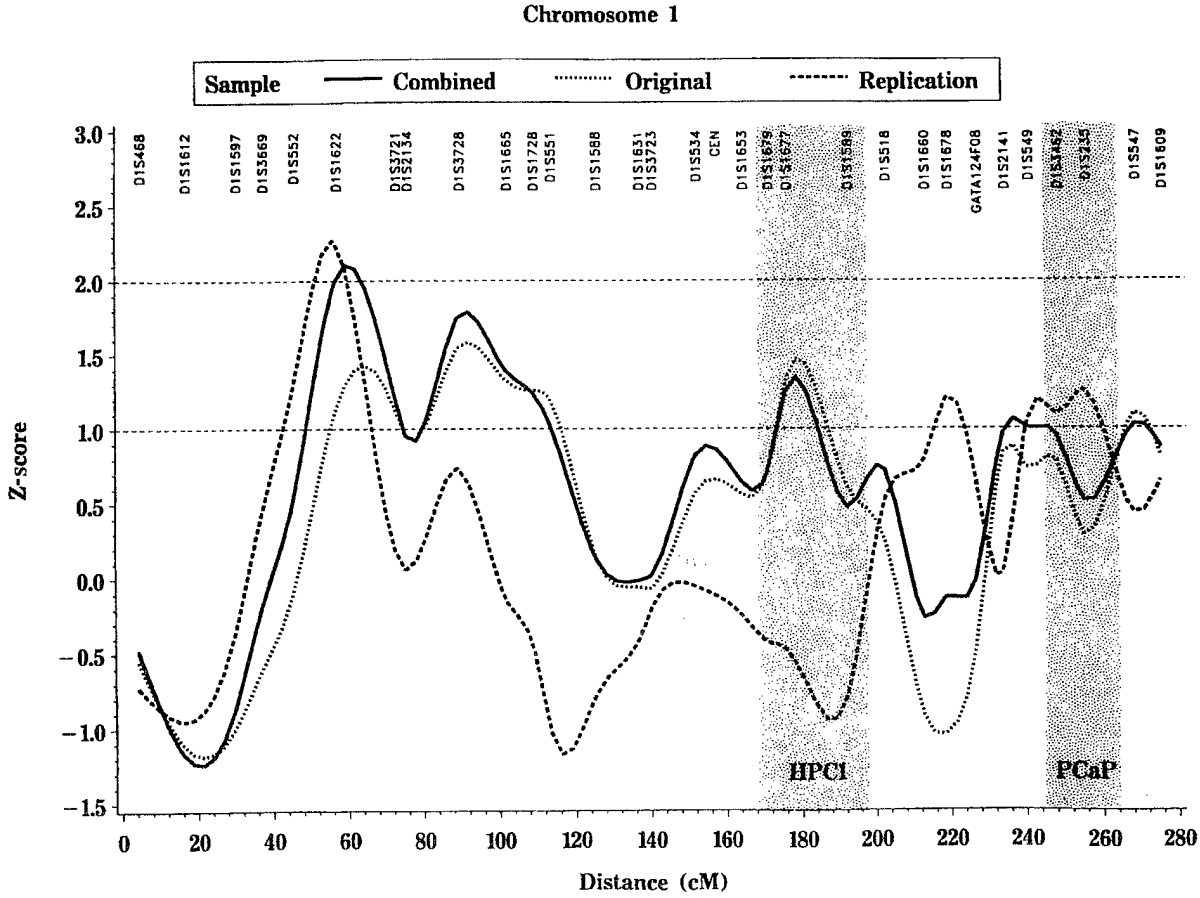

Fig. 1. Multipoint Z-scores for 30 chromosome 1 markers from the replication sample (N = 49 families), the original sample (N = 230 families), and the combined sample. Shaded regions indicate intervals alleged to contain HPC1 and PCaP.

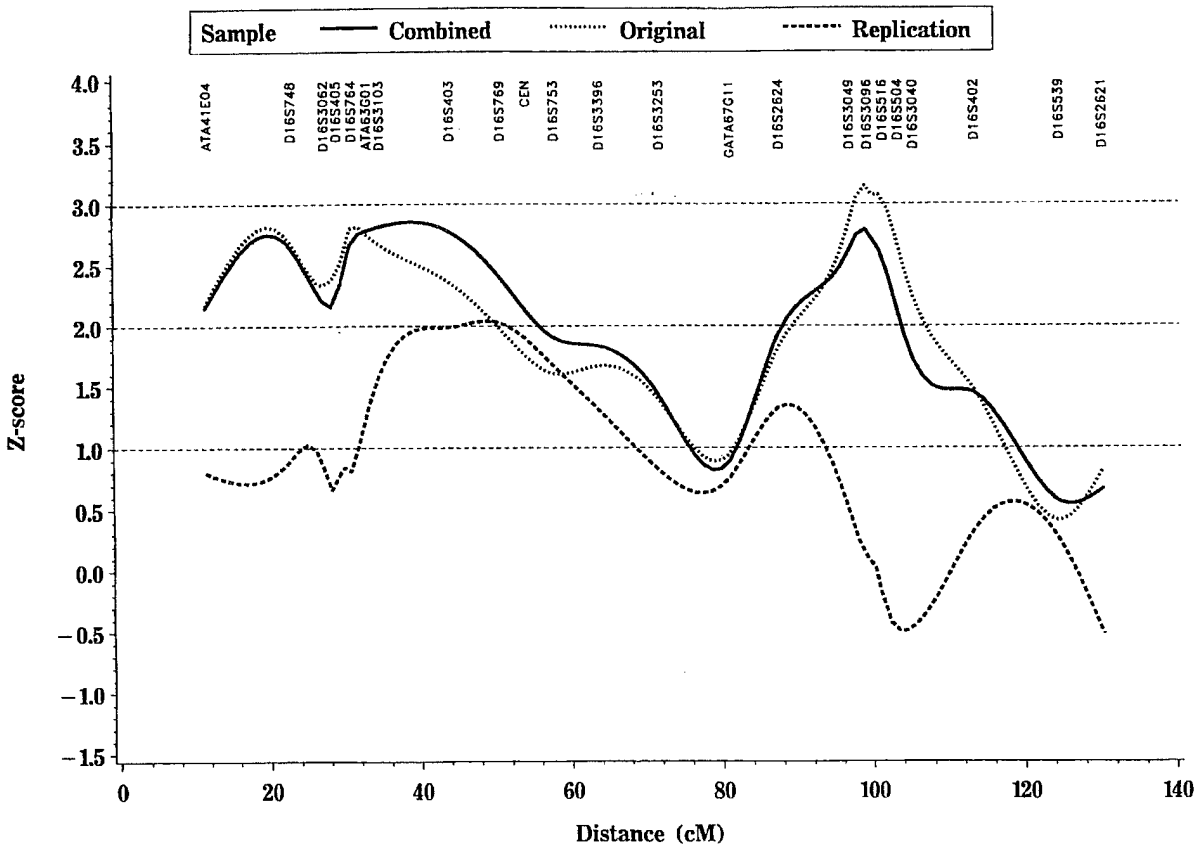

Fig. 2. Multipoint Z-scores for 22 chromosome 16 markers. Sample sizes are identical to those in Figure 1.

TABLE II. *P*-Values for Descriptive Categories and Their Associated Lod and Z-Scores Used to Inventory CaP Linkage Studies

Strength of evidence	Code[a]	*P*-value	Analysis method	
			Model-based	Model-free
Confirmatory	C	$p < 0.0001$	Lod > 2.97	Z > 3.70
Very strong	V	$0.0001 < p < 0.001$	2.97 > Lod > 2.07	3.70 > Z > 3.09
Moderate	M	$0.001 > p < 0.01$	2.07 > Lod > 1.18	3.09 > Z > 2.33
Weak	W	$0.01 < p < 0.05$	1.18 > Lod > 0.59	2.33 > Z > 1.65
Equivocal	E	$0.05 < p < 0.50$	0.59 > Lod > 0.00	1.65 > Z > 0.00
Negative	N	$p > 0.50$	Lod < 0.00	Z < 0.00

[a]Codes used to evaluate linkage evidence in Tables III and IV.

sample, the resultant Z-scores over this region lie between 0.5–1.0.

The results of the replication study of chromosome 16 are displayed in Figure 2. In our original genome scan, chromosome 16 gave the strongest evidence for linkage with two distinct signals: a broad region spanning approximately 39 cM on 16p, and a narrower region spanning approximately 17 cM on 16q [8]. The Z-scores for 16p in the replication sample are uniformly positive and attain nominal significance (i.e., $P < 0.05$) for the two most centromeric markers (D16S403 and D16S769). Multipoint Z-scores for the combined data are above 2.0 for the entire p arm.

The replication sample provides equivocal support for our earlier findings on 16q. While the multipoint Z-scores were positive for approximately 42 cM (from the centromere to D16S516), they fluctuated at about zero from D16S516 to the telomere. Again, owing to

TABLE III. Inventory of HPC1 Linkage Studies*

Reference	Partition	Family	Model type	Evidence					
				C	V	M	W	E	N
Smith et al. [6]	Total	91	P	•					
	Total	91	N	•					
Cooney et al. [16]	Total	59	N					•	
	FH+	20	N				•		
	FH−	39	N					•	
	AA	6	N					•	
Grönberg et al. [17]	US + Swedish	91	P	•					
	Swedish	12	P					•	
	US	79	P	•					
	US	79	N	•					
	US E-AOO	40	P	•					
	US E-AOO	40	N	•					
	US L-AOO	39	P					•	
	US L-AOO	39	N					•	
	US FH+	45	P	•					
	US FH+	45	N	•					
	US E-AOO and FH+	21	P	•					
	US E-AOO and FH+	21	N	•					
Hsieh et al. [18]	Total	92	N				•		
	E-AOO	46	N				•		
	L-AOO	46	N				•		
McIndoe et al. [19]	Total (Seattle)	49	P					•	
	Total (Hopkins)	49	P					•	
	Total	49	N					•	
	E-AOO	18	P						•
	L-AOO	28	P				•		
Eeles et al. [20]	Total	136	P					•	
	Total	136	N						•
	FH+	35	P					•	
	FH+	35	N					•	
	FH−	101	P						•
	FH−	101	N						•
Grönberg et al. [21]	Total	40	P					•	
	Total	40	N					•	
	E-AOO	12	P				•		
	E-AOO	12	N				•		
	L-AOO	28	P						•
	L-AOO	28	N					•	
Neuhausen et al. [22]	Total	41	P			•			
	1st Q	9	P			•			
	4th Q	10	P					•	
Berry et al. [23]	Total	144	P						•
	Total	144	N					•	
	E-AOO	67	N					•	
	FH+	47	N					•	
	MTM	102	N				•		
	E-AOO and FH+ and MTM	21	N					•	
Goode et al. [24]	Total	150	P					•	
	Total	150	N					•	
	E-AOO	66	P						•
	E-AOO	66	N						•
	L-AOO	78	P					•	
	L-AOO	78	N				•		

TABLE III. Continued

Reference	Partition	Family	Model type	Evidence C	V	M	W	E	N
	E-AOO and ≥5 aff	21	P					•	
	E-AOO and ≥5 aff	21	N					•	
	E-AOO and >1 gen	43	P					•	
	E-AOO and >1 gen	43	N					•	
Suarez et al. [8]	Total	230	N					•	
	E-AOO	115	N					•	
	L-AOO	115	N					•	
	FH+	111	N					•	
	FH–	119	N				•		
	CaB+	53	N					•	
Xu et al. [25]	New + old (total)	863	P	•					
	New	772	P			•			
	New MTM	491	P		•				
	Total MTM	550	P	•					
	New E-AOO and MTM	161	P		•				
	New L-AOO and MTM	330	P				•		
	Total E-AOO	306	P	•					
	Total FH+	224	P	•					
	New FH+	174	P				•		
	New FH–	598	P					•	
Gibbs et al. [26]	E-AOO	44	P				•		

*The interval includes reported markers between D1S1677 to D1S1660. The most "significant" marker was used to classify the evidence for linkage. FH+, family history positive; FH–, family history negative; AA, African American; US, United States; E-AOO, early age-of-onset; L-AOO, late age-of-onset; MTM, male-to-male transmission; 1st Q, lowest quartile of age-of-onset; 4th Q, highest quartile of age-of-onset; aff, affected; gen, generation; CaB+, positive family history of breast cancer. P, parametric analysis; N, model-free "nonparametric" analysis.

the larger size of the original sample, the combined families yield positive Z-scores for all of 16q, although the previous peak Z score of 3.15 at D16S3096 is decreased to 2.79 in the combined sample.

DISCUSSION

Ideally, a replication study should mimic as closely as possible the study design used to establish the original linkage. This is especially important for complex common disorders where there are likely many loci whose products interact with one another and with environmental factors to produce the disease phenotype [15]. Subtle differences in ascertainment, recruitment, diagnostic, or laboratory procedures may result in a failure to replicate. When important differences exist between linkage studies, failure to replicate does not necessarily mean that the original linkage claim was in error. A susceptibility locus may be segregating in population A and not in population B. Accordingly, failure to replicate a linkage in population B should not be taken as evidence against the initial linkage because (unknown to the investigator) there is no power to detect the susceptibility locus in a sample drawn from population B.

In practice, replication studies are never identical to the original study. The sample of families analyzed in this report, for instance, is smaller than that used to map either HPC1 [6] or PCaP [7], and it is smaller than our own original sample of multiplex sibships [8]. Moreover, whereas our original sample of 230 families was ascertained through probands from a single site (WUMS), our replication sample derives from three sites. Thus, while all of the families reported here are of European descent, and no between-site differences in mean age-of-onset or mean Gleason grade were found, it is possible that unrecognized differences are present that could affect the linkage results.

We have inventoried all CaP linkage studies published to date and have scored the evidence they present with respect to HPC1 and PCaP, using descriptors based on the *P*-values of the reported linkage statistics (Table II). Not all of the published studies are independent, due to sample overlap between different reports. Table III evaluates the evidence for the interval alleged to contain HPC1 [6,8,16–26], and Table IV

TABLE IV. Inventory of PCaP Linkage Studies*

Reference	Partition[a]	Family	Model type[b]	Evidence C	V	M	W	E	N
Berthon et al. [7]	Total	47	P		•				
	Total	47	N		•				
	E-AOO	9	P			•			
	E-AOO	9	N	•					
Whittemore et al. [27]	Total	97	P						•
	E-AOO	48	P					•	
	L-AOO	49	P					•	
Gibbs et al. [28]	Total	152	P					•	
	Total	152	N					•	
	E-AOO	20	P				•		
	E-AOO	20	N					•	
	FH+	46	P				•		
	FH+	46	N					•	
Berry et al. [23]	Total	144	P						•
	Total	144	N						•
	E-AOO	67	N						•
	FH+	47	N					•	
	MTM	102	N						•
	E-AOO and FH+ and MTM	21	N					•	
Suarez et al. [8]	Total	230	N					•	
	E-AOO	115	N						•
	L-AOO	115	N				•		
	FH+	111	N					•	
	FH–	119	N						•
	CaB+	53	N					•	
Gibbs et al. [26]	Total	94	P			•			
	Total	94	N					•	
	E-AOO	44	P			•			
	E-AOO	44	N					•	
	L-AOO	50	P				•		
	L-AOO	50	N						•

*The interval includes reported markers between D1S235 to D1S1609. The most "significant" marker was used to classify the evidence for linkage.
[a]See footnote to Table III.
[b]See footnote to Table III.

assesses the evidence for the interval alleged to contain PCaP [7,8,23,26–28]. Some studies reported results assuming a parametric model: either the model obtained by Carter et al. [3] from segregation analysis, or a closely related model that incorporates the age-dependent penetrance features of the model of Carter et al. [3]. When a linkage signal is detected with a parametric model, most studies estimate the proportion of linked families with the HOMOG [29] or ANALYZE [30] programs. Other studies have used a model-free approach, usually computing nonparametric linkage statistics (NPL Z-scores) obtained with GENEHUNTER [13] or GENEHUNTER-PLUS [14]. A number of the studies inventoried in Tables III and IV report results from both approaches.

Without exception, all studies published since the original report by Smith et al. [6] have also presented linkage analyses for various subsets of their data, and these are reported in Tables III and IV. Families are usually partitioned according to some function of the affected members' mean age-of-onset (e.g., a median split, youngest quartile vs. oldest quartile, or onset before age 65 vs. onset after age 65), or according to the density and distribution of CaP cases (e.g., family history positive vs. family history negative, a male-to-male transmission pattern, or an X-linked pattern), or a combination of these partitioning variables.

The heterogeneity seen in Tables III and IV underscores the difficulty encountered when attempting to verify a linkage claim for a complex phenotype. Inconclusive replication is by no means a phenomenon peculiar to prostate cancer linkage studies.

Over the critical 1q24–25 region, our replication sample did not give any evidence for increased allele sharing among affected relatives. Allele sharing shows an increase above its null value at marker D1S518 (1q25.2), but this marker is outside the narrow region believed to harbor HPC1. A nonsignificant increase in allele sharing for the PCaP region, however, was obtained in the replication sample.

Although the present replication sample is too small to partition for any meaningful subgroup analyses, it is perhaps noteworthy that the region of greatest allele sharing on chromosome 1 occurs at D1S1622 (Z = 2.26), which is the same location (1p35.1) as our previous strongest signal (Z = 3.78) in multiplex CaP families that also reported a positive family history of breast cancer [8].

The attempt to replicate our earlier chromosome 16 findings met with mixed results. The replication sample gave positive Z-scores for all p-arm markers. Since the original sample was 4.69 times larger than the replication sample, we would expect the replication Z-scores to be only $1/(4.69)^{1/2} = 0.462$ times as large, assuming that the "signal" is equally strong in the replication sample. The mean ratio of the replication Z-scores to those obtained from the original sample is 0.466 for the nine chromosome 16 p arm markers. This close agreement is consistent with replication. The signal remains very diffuse, however, with no discernible peak in the Z-score distribution in the combined sample that would allow sublocalization of a putative susceptibility locus.

As noted above, replication of our previous findings for 16q is mixed. Adjusting for the smaller size of the replication sample reveals that the five most centromeric markers (covering an interval of approximately 30 cM) actually provide stronger evidence than obtained in the original sample. But for the 16q23–24 region, where our highest Z-scores were originally localized, the replication sample is equivocal to negative. The combined Z-score remains nominally significant ($P < 0.003$).

CONCLUSIONS

A model-free linkage analysis of 45 new and 4 expanded multiplex families lends no support for the existence of HPC1 and is equivocal with respect to PCaP. In the combined sample of 275 families, however, both the HPC1 and the PCaP regions continue to reflect a modest excess of allele sharing as evidenced by the positive, albeit nonsignificant, Z-scores. Mixed support for our earlier finding of two signal regions on chromosome 16 was obtained in the replication sample. Support remains strong for the existence of a susceptibility locus on 16p, but its subregional localization is poorly delineated. We are unable to verify our strongest previous linkage signal at 16q23–24, but the region immediately proximal to 16q23 continues to reflect increased allele sharing among affected family members.

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Genomewide Scan for Prostate Cancer–Aggressiveness Loci

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The aggressiveness of prostate cancer (PCa) varies widely: some tumors progress to invasive, potentially life-threatening disease, whereas others stay latent for the remainder of an individual's lifetime. The mechanisms resulting in this variability are not yet understood, but they are likely to involve both genetic and environmental influences. To investigate genetic factors, we conducted a genomewide linkage analysis of 513 brothers with PCa, using the Gleason score, which reflects tumor histology, as a quantitative measure of PCa aggressiveness. To our knowledge, this is the first time that a measure of PCa aggressiveness has been directly investigated as a quantitative trait in a genomewide scan. We employed a generalized multipoint Haseman-Elston linkage-analysis approach that regresses the mean-corrected cross product between the brothers' Gleason scores on the estimated proportion of alleles shared by brothers identical by descent at each marker location. Our results suggest that candidate regions on chromosomes 5q, 7q, and 19q give evidence for linkage to PCa-aggressiveness genes. In particular, the strongest signals detected in these regions were at the following markers (with corresponding *P* values): for chromosome 5q31-33, between markers D5S1480 and D5S820 ($P = .0002$); for chromosome 7q32, between markers D7S3061 and D7S1804 ($P = .0007$); and, for chromosome 19q12, at D19S433 ($P = .0004$). This indicates that one or more of these candidate regions may contain genes that influence the progression of PCa from latent to invasive disease. Identification of such genes would be extremely valuable for elucidation of the mechanism underlying PCa progression and for determination of treatment in men in whom this disease has been diagnosed.

Introduction

Prostate cancer is the most common neoplasm among men in the United States: in the year 2000, ~180,400 men in the United States will receive a diagnosis of prostate cancer (PCa [MIM 176807]), a disease accounting for ~31,900 deaths annually (Greenlee et al. 2000). This disease will be diagnosed in ~15% of men in the United States, and the results of autopsy studies suggest that 30% of men of age >45 years may have prostate lesions that are histologically identifiable as PCa (Dhom 1983; Kosary et al. 1995). While a good number of these lesions will remain latent for a man's lifetime, little is currently known about what makes some PCa biologically aggressive and more likely to progress to metastatic and potentially lethal disease. One compelling possibility is that genetic factors help drive the mechanisms underlying PCa aggressiveness.

A key pathological measure of aggressiveness is the Gleason score (Gleason 1992) assigned to a prostate tumor. The Gleason score reflects the patterns of tissue architecture observed by a pathologist in two prostate biopsy or surgery samples. Each pattern is given a whole-number score between 1 and 5, so the total Gleason-score range is 2–10. For tissue with heterogeneous scores, the maximum two scores are summed to obtain the total score. Low Gleason scores (i.e., 2–4) indicate that the tumor cells are well differentiated and are organized into glandular structures. In contrast, higher scores (i.e., 8–10) signify less differentiation of the tumor cells: they appear solid and dissipate together. Poor differentiation and, hence, a high Gleason score, is a strong prospective gauge of which tumors will penetrate the prostate capsule, invade the seminal vesicles, and spread to the lymph nodes. Therefore, genes linked to Gleason score and, thus, tissue architecture could serve as molecular markers of PCa aggressiveness. In addition, such genes may increase our knowledge about the biological mechanisms underlying the progression of PCa from latent to invasive disease.

Previous work searching for genetic markers of PCa

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aggressiveness has included loss-of-heterozygosity (LOH) and candidate-gene approaches (Ware 1994; Nupponen and Visakorpi 1999). Genomic regions that have been shown to contain LOH and thus, possibly, tumor-suppressor genes for more-aggressive PCa include those on chromosomes 5, 7, 10, and 16 (Takahashi et al. 1994; Oakahashi 1995; Cunningham et al. 1996; Komiya et al. 1996; Elo et al. 1997; Elo et al. 1999). Other work has detected associations between PCa aggressiveness and variants in numerous candidate genes, including *CYP3A4* (Rebbeck et al. 1998; Paris et al. 1999), *PTEN* (McMenamin et al. 1999), the androgen receptor (Giovannucci et al. 1997), and the vitamin D receptor (Ingles et al. 1997). In contrast with these studies of tumor aggressiveness, linkage analyses have focused on the detection of regions containing susceptibility genes—that is, those genes implicated in the development of PCa. Several regions that have been identified by linkage analysis may contain genes for PCa development at chromosomal locations 1q24-25 (*HPC1* [MIM 601518]) (Smith et al. 1996), 1q42.2-43 (*HPC2* [MIM 602759]) (Berthon et al. 1998), 1p36 (*CAPB* [MIM 603688]) (Gibbs et al. 1999), and Xq27-28 (*HPCX* [MIM 300147]) (Xu et al. 1998). We have recently undertaken a sib-pair genomewide linkage analysis that detected another PCa-susceptibility locus at chromosome 16q (Suarez et al. 2000), a region showing high levels of LOH (Carter et al. 1990*a*; Elo et al. 1997; Latil et al. 1997; Elo et al. 1999; Paris et al., in press).

To our knowledge, however, no genomewide scan has been done to search for PCa-aggressiveness genes. Therefore, in the present paper, we give results from a linkage analysis of markers located throughout the genome, to determine whether they are inherited along with a predisposition to more-aggressive PCa, as indicated by Gleason score.

Material and Methods

Subjects

Probands were recruited into this study from urology practices in St. Louis, Missouri, and Cleveland, Ohio, as well as from general referrals to the study. The probands' self-reported family history of PCa was used as a guide for recruitment of their affected brothers. In the present analysis, a total of 513 men from concordant sibships (i.e., two or more brothers with PCa) in which at least two brothers had information available on their Gleason scores were recruited into the study and were genotyped. A total of 189 families had two affected brothers, 41 families had three affected brothers, two families had four affected brothers, and one family had two pairs of affected brothers who were cousins (for the equivalent of 326 sib pairs). In addition to 48 new subjects, this sample includes the 465 men who were used in our recent genomewide scan of susceptibility genes and who had complete Gleason-score information. The median age at diagnosis among these men was 65 years (range 42–91 years). Further details about the original subjects are given in our previous report (Suarez et al. 2000).

We use Gleason score as a measure of tumor aggressiveness, since, as noted above, it is generally considered to be a strong predictor of survival with PCa (Gleason 1992). Our collaborating pathologists ascribed Gleason scores to biopsy specimens and, when such specimens existed, to radical-prostatectomy specimens. For the present analyses, we used the Gleason score from the prostatectomy specimen whenever available (78% of all samples). If it was not available, we used the Gleason score from the biopsy specimen. The institutional review boards of Washington University and University Hospitals of Cleveland approved this study, and all subjects gave informed consent to take part in the project.

Genotyping

DNA was extracted from the study subject's blood, by use of standard methods, and was sent to the Center for Medical Genetics, Marshfield Medical Research Foundation for genotyping. Samples were typed using the Marshfield Screening Set 9 (Yuan et al. 1997). With use of this screening set, 364 autosomal simple-tandem-repeat polymorphisms, which are spaced at ~9-cM intervals across the genome (sex-equal maps) and which have an average heterozygosity of 77% (Broman et al. 1998), were genotyped. We used these markers to confirm reported relationships among the sib pairs, using RELTEST (S.A.G.E. 2000). Resulting exclusions in the original data are given elsewhere (Suarez et al. 2000). In the new samples with complete Gleason-score information, we detected one pair of monozygotic twins, which was excluded from our linkage analysis.

Statistical Analysis

For our statistical analysis, we used a multipoint generalized Haseman-Elston (HE) linkage test (Elston et al. 2000; S.A.G.E. 2000). In particular, we used linear regression in which the dependent variable—the mean-corrected cross product (i.e., of Gleason scores) between brothers—is regressed on the estimated proportion of alleles at a particular marker that are shared among brothers identical by descent (IBD), which is denoted as π_j for sib pair j. The form of the model is as follows:

$$E(x_{1j} - \mu : x_{2j} - \mu) = \alpha + \beta\hat{\pi}_j , \qquad (1)$$

where x_{ij} is the Gleason score for individual i in sib pair j and where μ is the population mean of all Gleason

scores. Since Gleason scores only exist for men with PCa, we estimated μ from the mean Gleason score in our sample. The expected value of the dependent variable is equal to the sibling covariance. In the presence of linkage, sib pairs that share the region—that is, those sib pairs with high π_j values—are expected to have highly correlated Gleason scores, which imply high sib covariance. In contrast, sib pairs that do not share the region—that is, those sib pairs with low π_j values—are expected to have less-correlated Gleason scores and low sib covariance. The regression coefficient β can be written as follows:

$$\beta = (1 - 2\theta)\sigma_g^2 , \quad (2)$$

where θ is the recombination fraction and where σ_g^2 equals the total genetic variance (Elston et al. 2000). From (2) we see that, in the presence of tight linkage (i.e., $\theta \cong 0$), β estimates the genetic variance. Thus, values of β that are statistically significantly >0 suggest linkage. The intercept α equals the residual sibling covariance. S.A.G.E. (2000) uses a generalized least-squares approach to fit model (1), estimating a covariance matrix that accounts for the correlation among the residuals.

We also reanalyzed the data by use of the original HE approach, which regresses the squared difference of brothers' Gleason scores on the estimated proportion of alleles shared IBD (Haseman and Elston 1972; S.A.G.E. 2000). This approach has been widely used in the past, and, although it is generally less powerful than the new HE approach, there are situations where it is more powerful than the new HE method (Palmer et al. 2000).

Because of the pathological criteria used in the assignment of Gleason scores, unit increases in scores might not represent equidistant changes in cellular differentiation. For example, the increase in Gleason score from 5 to 6 likely represents a smaller change in differentiation than does an increase from 6 to 7. This is because the two values resulting in a Gleason score of 7 are usually 3 and 4, and a value of 4 is given only if there is relatively poor tumor differentiation. Instead of the ordinal ranking of Gleason scores used here, one could instead combine scores into the following four groups, to indicate similar levels of differentiation: (*a*) 2–4 (well differentiated); (*b*) 5 and 6 (moderately differentiated); (*c*) 7 (less moderately differentiated); and (*d*) 8–10 (poorly differentiated) (Sakr and Grignon 1997; Stanford et al. 1999). Therefore, we repeated our analyses by combining Gleason scores in this manner, treating the midpoints of the categories (i.e., 3, 5.5, 7, and 9) as a continuous variable.

For sibships with more than two brothers, we assumed that all pairs were independent, since estimates of π_j are pairwise independent under the null hypothesis of no linkage (Hodge 1984). Allele frequencies were calculated by random selection of one individual from each family.

Results

The median Gleason score among the men in the present study was 6 (range 2–10). The frequency distribution of Gleason scores was as follows: Gleason 2 (1%), Gleason 3 (9%), Gleason 4 (7%), Gleason 5 (26%), Gleason 6 (29%), Gleason 7 (20%), Gleason 8 (4%), Gleason 9 (3%), and Gleason 10 (.2%).

Figure 1 presents the multipoint linkage results from the new and original HE approaches across all chromosomes (except the X and Y chromosomes). The vertical axis of each chromosome's plot gives the $-\log(P$ value) from the t test of the departure of β from zero, with 324 df (number of affected sib pairs [326] minus the number of parameters estimated [2—that is, α and β]) (S.A.G.E. 2000). The dotted horizontal lines indicate where $P < .001$ ($-\log[.001]=3$). With use of the new HE approach, we found evidence ($P < .001$) for linkage with Gleason score in three regions on chromosomes 5q, 7q, and 19q. On chromosome 5q, we observed a relatively broad region (~26 cM in length) within which $P < .001$ was maintained and a peak ($P = .0002$) approximately halfway between markers D5S1480 (5q31.3) and D5S820 (5q33.3). For chromosome 7q, $P < .001$ for an ~8-cM region, with a peak ($P = .0007$) ~2 cM centromeric to D7S1804 (7q32.3). On chromosome 19q, $P < .001$ was observed across ~5 cM, with a peak ($P = .0004$) at D19S433 (19q12). In addition to these three regions, chromosomes 10 and 18 exhibited intriguing, albeit statistically weaker, peaks at D10S1248 ($P = .0012$) and 2 cM centromeric to D18S976 ($P = .0024$).

The original HE approach indicated potential regions of linkage similar to those seen with the new HE approach, although generally with statistically weaker results (fig. 1, *broken lines*). In particular, for the three chromosomal regions demonstrating linkage with $P < .001$, the original HE approach gave the following peak P values: chromosome 5q, $P = .0053$; chromosome 7q, $P = .0076$; and chromosome 19q, $P = .0088$. This result is not surprising, in light of the increased power that is potentially available with use of the new HE approach; this increased power is especially apparent when the residual correlation is limited (Elston et al. 2000; Palmer et al. 2000). With use of the original HE approach, chromosomes 1p and 9q also demonstrated slight linkage to Gleason score ($P < .01$), with peaks between D1S1622 and D1S3721 ($P = .003$) and at D9S930 ($P = .008$).

Table 1 gives additional details for the Gleason-score linkage regions on chromosomes 5q, 7q, and 19q. In particular, for markers within and bordering these

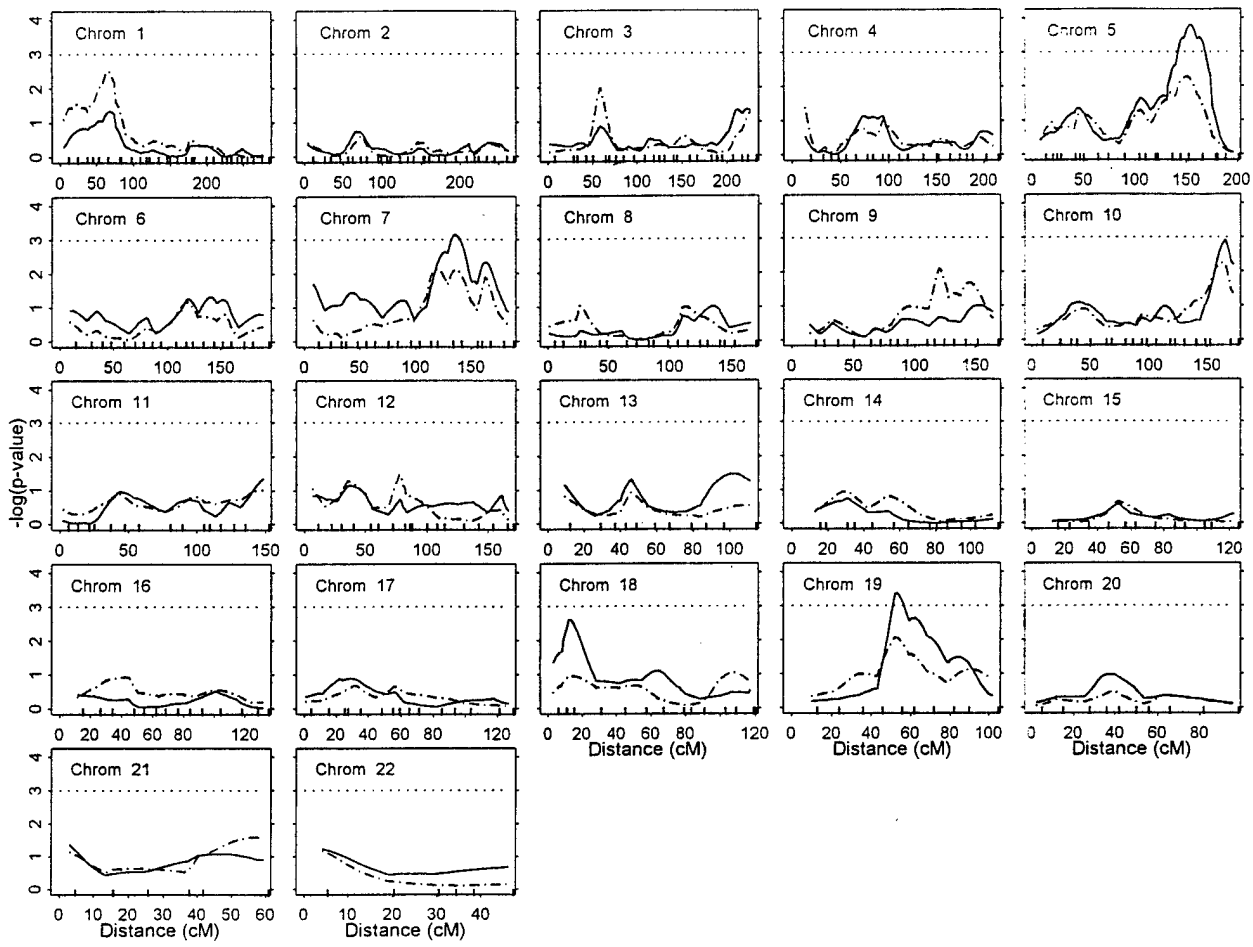

Figure 1 Results from a genomewide scan of PCa-aggressiveness genes; Gleason score was used as a quantitative trait. Unbroken lines denote results from the new HE analysis; broken lines denote results from the original HE analysis. Tick marks across horizontal axes indicate marker locations (in cM) (as specified on the Center for Human Genetics, Marshfield Medical Research Foundation Web site). *P* values are from a *t* test of the HE regression coefficient, with 324 df (number of affected sib pairs [326] minus the number of parameters [2]). Dotted horizontal lines indicate where $P = .001$ ($-\log[P] = 3$); thus, for peaks above these lines, $P < .001$.

regions, intermarker spacing, regression coefficients from the new HE analysis (plus their standard errors), and corresponding *P* values are presented. The regression coefficients indicate that, in each region, the genetic variance accounted for by the linkage is slightly greater than one Gleason-score unit. Furthermore, low *P* values extend across relatively broad regions on these chromosomes, providing additional support for the presence of genes that influence the aggressiveness of PCa (Terwilliger et al. 1997).

When the four groups of Gleason scores reflecting tumor differentiation were used, the minimum (new) HE *P* values indicated slightly more support for linkage on chromosome 5q31.3-33.3 but somewhat less evidence for linkage on chromosomes 7q32.3 and 19q12. In particular, for chromosome 5q, the strongest signal now gave $P = .00008$, with the peak shifted 4 cM telomeric to the original peak. For chromosome 7q, $P = .0048$ (shifted 2 cM centromeric to the original peak). For chromosome 19q, peak $P = .013$ (same location). The larger *P* values for chromosomes 7q and 19q are not necessarily unexpected, because combining scores reduces variation crucial to the power of our linkage analysis. Even with this recoding of the Gleason scores, our results continue to suggest linkage to three regions that might contain PCa-aggressiveness genes. Use of different allele frequencies (e.g., equal across all markers) did not materially alter our results (not shown).

Discussion

The results of the present study give evidence ($P < .001$) that loci on chromosomes 5q31.3-33.3, 7q32.3, and 19q12 might contain genes linked to Gleason score,

Table 1

Multipoint Results from the New HE Analysis, for Regions on Chromosomes 5q, 7q, and 19q That Show Linkage with Gleason Score (i.e., with Peak $P < .001$).

Chromosome and Marker[a]	Intermarker Distances[b] (cM)	β[c]	Standard Error of β	P[d]
5q:				
D5S816	8.16	1.11	.40	.0025
D5S1480	12.28	1.32	.39	.0004
D5S820	12.36	1.37	.41	.0004
D5S1471		1.06	.40	.0046
7q:				
D7S3061	8.54	1.12	.40	.0026
D7S1804	12.95	1.21	.38	.0008
D7S1824		.80	.39	.0190
19q:				
D19S714	9.60	.26	.44	.2781
D19S433	6.81	1.37	.41	.0004
D19S245	9.39	1.14	.41	.0029
D19S178		.94	.39	.0085

[a] All markers for which $P < .01$ or that border the peaks are shown and are listed centromeric to telomeric from top to bottom.

[b] Spacing between contiguous markers in genome scan. Distances shown are between the corresponding marker and the marker listed immediately below.

[c] Regression coefficient from HE model (1).

[d] From a t test with 324 df (number of affected sib pairs [326] minus number of parameters [2]).

which is a measure of PCa aggressiveness. Recent LOH and candidate-gene work lends some additional support to the possibility that PCa-aggressiveness genes reside within or near these candidate regions. An association between LOH and tumor-node-metastasis stage has been detected on the border of the chromosome 5q candidate region (Cunningham et al. 1996). The candidate gene α-catenin is also located near this region. This gene is part of the E-cadherin pathway and has been associated with PCa aggressiveness in numerous studies (Ewing et al. 1995; Richmond et al. 1997; Umbas et al. 1997; Morita et al. 1999). LOH in PCa has also been reported in a region (chromosome 7q31.1) near the candidate locus that we have detected on chromosome 7q (Zenklusen et al. 1994; Latil et al. 1995; Cunningham et al. 1996). Recent work has also found associations between LOH and Gleason score on chromosome 7 (Takahashi et al. 1994; Oakahashi 1995). To our knowledge, there has been no LOH or candidate-gene work near the linkage region on chromosome 19.

In addition to primary linkage on chromosomes 5q, 7q, and 19q, we detected weaker ($.001 < P < .01$) signals on chromosomes 1p, 9q, 10q, and 18p. The chromosome 1p region was also linked to PCa susceptibility in our earlier work (Suarez et al. 2000) among those with a positive history of breast cancer, and it is in close proximity to the *CAPB* locus (Gibbs et al. 1999). If there is a single gene driving all of these results, *CAPB* may also contribute to PCa aggressiveness. For chromosomes 9q, 10q, and 18p, the peak regions are relatively narrow, primarily reflecting linkage only between the peak markers and Gleason score (fig. 1).

Although none of the results reported in the present study fulfill the alpha-level criterion for "significant" linkage (Lander and Kruglyak 1995), the magnitude of the results may simply reflect sample-size issues in our sib-pair study as well as the potential for numerous genetic and environmental factors interacting in the multistep progression of PCa from latent to aggressive disease (Witte et al. 1996).

This multistep process—whereby numerous malignant events are required for a normal cell to transform into a malignant cancer cell (Carter et al. 1990*b*)—provides the biological rationale for why a gene might be linked to PCa aggressiveness but not to PCa development. The multiple steps appear to be driven by different regulating factors that distinguish which cancers progress to advanced, lethal disease (Isaacs et al. 1995; Hayward et al. 1998). More specifically, some factors may initiate the development of PCa, whereas others may affect progression from relatively indolent to invasive disease. In the present study, we have focused on genetic loci that may regulate one of the latter steps in PCa: progression to aggressive disease. More specifically, since Gleason score reflects the potential progression of PCa to metastatic and lethal disease, the regions detected in this report could harbor tumor-aggressiveness genes. In contrast, previous linkage analyses of PCa have searched for chromosomal regions that may contain genes that regulate an earlier step in the process: initiation of disease. For example, we have previously detected a region on chromosome 16q23 that may harbor a PCa-susceptibility gene (Suarez et al. 2000). Since the steps for initiation and progression may be unique, it is not surprising that mostly different loci were detected in our present and previous reports. While the discovery of susceptibility genes will be valuable for screening purposes, aggressiveness genes will provide important information about the most-appropriate treatment among men in whom PCa has already been diagnosed. Since most of the men (78%) we studied had radical prostatectomies, any aggressiveness genes within the loci reported here would be primarily relevant to men with localized PCa diagnosed at a younger age (i.e., those men amenable to having a prostatectomy). Nevertheless, such patients with PCa constitute a large proportion of men with the disease who need important information about the most adequate course of treatment.

A benefit of most of our subjects having undergone prostatectomies is that the resulting specimens pro-

vide the most accurate measure of Gleason score. In contrast, a Gleason score from a needle-biopsy specimen may underestimate the true score. Differential misclassification of Gleason scores between siblings—arising from their having different treatments and, thus, different specimens—will, on average, decrease the magnitude and statistical significance of the HE regression coefficients (Ott 1999). To increase the coefficient's magnitude and statistical significance, the degree of Gleason-score misclassification would have to be related to the brothers' IBD sharing at the corresponding marker allele. We have investigated the impact of misclassification by statistically adjusting our linkage analysis for whether sibling pairs were concordant or discordant for PCa treatment (66% of the pairs were concordant). In particular, in a subsequent linkage analysis, we have used the conventional adjustment approach of inclusion of a covariate indicating treatment concordance (or discordance) between pairs of siblings. This adjustment left our results fundamentally unchanged, and we observed no linkage between PCa treatment and IBD sharing of marker alleles at the putative aggressiveness loci. Therefore, any misclassification resulting from different types of specimens used in the measurement of Gleason score do not appear to have affected our findings. Furthermore, any misclassification would lead to our results underestimating the actual linkage. Another potential source of misclassification is the fact that a number of different pathologists assigned Gleason scores to the specimens. Our past experience reviewing thousands of cases of prostate cancer indicates that the scores are reproducible 70% of the time and that they otherwise disagree by no more than one unit. More importantly, it is inconceivable that misclassification by different pathologists would be associated with the brothers' IBD sharing of marker alleles at the aggressiveness loci. Therefore, any misclassification of the brothers' Gleason scores arising from different pathologists would, at most, lead to a limited underestimation of the true linkage.

In light of the expansion of prostate-specific–antigen (PSA) screening during part of this project's recruitment period (i.e., in the early to mid-1990s), the potential impact of this diagnostic tool on our results merits consideration. Our primary concern is that, within brother pairs, earlier detection resulting from PSA screening could result in one brother having a Gleason score that is lower than that which would have been observed prior to the widespread use of PSA tests. Therefore, any bias resulting from PSA screening will reflect whether the brothers' diagnoses were made within concordant or discordant PSA-screening eras (i.e., pre- and post-PSA eras) and whether PSA era is linked to the brothers' IBD sharing of alleles. We investigated this possibility by undertaking an adjusted linkage analysis that incorporated a covariate indicating whether they both had been given a diagnosis in the same PSA era, using 1994 as a cutoff point for when PSA was fully established as a screening tool (63% were concordant for PSA-screening era). We observed no notable differences in the linkage peaks and found that PSA-screening era is not linked to IBD sharing of alleles at these aggressiveness loci. (Using 1992 as a cutoff point for establishment of PSA screening gave similar results.) This indicates that the introduction of PSA testing does not appear to have had any impact on our linkage results.

In summary, our novel genomewide scan of Gleason score has detected loci on chromosomes 5q31.3-33.3, 7q32.3, and 19q12 that might harbor PCa-aggressiveness genes. Detection of such genes may provide important insights into the underlying mechanism driving the progression of PCa from latent to invasive disease. Moreover, knowledge about aggressiveness genes could help guide treatment plans among men with diagnosed PCa.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/ (for all markers used here)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for PCa [MIM 176807], *HPC1* [MIM 601518], *HPC2* [MIM 602759], *CAPB* [MIM 603688], and *HPCX* [MIM 300147])

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Identification and Fine Mapping of a Region Showing a High Frequency of Allelic Imbalance on Chromosome 16q23.2 That Corresponds to a Prostate Cancer Susceptibility Locus[1]

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ABSTRACT

Linkage to a prostate cancer susceptibility locus was recently reported on chromosome 16q23. We now report a region exhibiting a high frequency of allelic imbalance (AI) corresponding to this locus in tumors from 51 men diagnosed with prostate cancer using the same linked markers. The highest frequency of AI was found at markers *D16S3096* (45%) and *D16S516* (53%) that map to chromosome 16q23.2. In addition, 19 of the 51 (37%) prostate tumors showed interstitial AI involving one or both of these markers. This result strongly suggests that a candidate prostate cancer tumor suppressor gene maps between markers *D16S3096* and *D16S516*. We estimate that the distance between these markers is approximately 118 kb using a Stanford radiation hybrid panel. We observed a positive association with family history ($P = 0.048$) when comparing those men showing interstitial AI at markers *D16S3096* and/or *D16S516* with those without any imbalance at these two markers. Taken together, these data suggest that we have precisely localized a region of chromosome 16q23.2 that may harbor a prostate cancer tumor suppressor gene implicated in the development of non-familial and possibly familial forms of prostate cancer.

INTRODUCTION

Prostate cancer is the second leading cause of cancer-related deaths among men in the United States (1). Unfortunately, the molecular pathogenesis of this disease remains poorly understood. Epidemiological data suggest a strong familial component, and it has been estimated that 9% of all prostate cancers occurring by age 85 years are the result of a hereditary predisposition (2). This frequency rises to 43% of early-onset (age 55 years or younger) prostate cancer cases (2). A hereditary prostate cancer susceptibility locus termed HPC1 was mapped to chromosome 1q24-q25 using a genome-wide scan of families that each had three or more affected first-degree members (3). Further analysis and expansion of these data suggested that linkage to HPC1 may be restricted to families with early-onset prostate cancer (4, 5). There is also some suggestion that linkage to this region may be positively affected by the inclusion of African-American prostate cancer families (6). Whereas a modest familial association with this region has been supported by two independent studies (6, 7), three other studies have been unable to confirm linkage (8–10). These data suggest that HPC1 may account for some, but not all, hereditary prostate cancer cases.

Another locus on chromosome 1q42.2-q43 has recently been reported to show linkage in French and German prostate cancer families (10), but this has not been confirmed in independent studies (11, 12). Linkage to a third prostate cancer susceptibility locus on chromosome 1 at 1p36 that may also be associated with increased risk of brain cancer has recently been reported (13). In addition, a locus on chromosome X has also been reported (14). There have not yet been any independent reports to confirm the latter associations. This ambiguity in linkage may reflect considerable heterogeneity in hereditary prostate cancer, with individual loci accounting for only a small fraction of the hereditary prostate cancer population.

In a recent study of 504 brothers with prostate cancer from 230 multiplex sibships involving two authors of this study (J. S. W. and W. J. C.), a positive linkage to chromosomes 2q, 12p, 15q, 16p, and 16q was reported (15). The strongest association was at chromosome 16q23. Frequent deletions of 16q have been reported in prostate cancer, and the literature suggests that this region may harbor at least three tumor suppressor loci (16–25). LOH[3] on 16q has also been reported in other cancers, including breast cancer (26), Wilms' tumors (27), and hepatocellular carcinoma (28). These data imply that one or more genes on 16q23 may be implicated in the development of prostate cancer and other malignancies. To date, no tumor suppressor genes have been identified from this region.

In this study, we sought to provide further evidence for a prostate cancer susceptibility gene on chromosome 16q23 and to further delineate this region using AI approaches. We now report a high frequency of AI in prostate tumors within the region on chromosome 16q23 corresponding to that identified in our sibling pair studies. We have further narrowed this region to approximately 118 kb by identifying tumors with interstitial loss. Our studies provide strong evidence that this region harbors a prostate cancer tumor suppressor gene that may be inactivated in a significant number of familial and nonfamilial forms of prostate cancers.

MATERIALS AND METHODS

Patient Selection and Tissue Evaluation. A series of 55 prostate cancer patients who underwent radical prostatectomy between the years of 1991 and 1998 at the Cleveland Clinic Foundation was identified through the Cleveland Clinic Foundation's Tumor Registry. Clinical characteristics, including Gleason grade, PSA, and tumor-node-metastasis (TNM) stage, and other potentially important factors, such as age at diagnosis, were obtained from medical records and are presented in Table 1. All tumors were graded according to the Gleason system (29). The tumor stage was determined after review of the microscopic sections from the surgical specimen (30). The mean age at the time of diagnosis was 61 years (age range, 47–73 years). The study design was approved by the Institutional Review Board of the Cleveland Clinic Foundation.

Tissue Microdissection and DNA Extraction. For each patient, a paraffin-embedded tumor tissue block was chosen such that both normal and tumor tissue were present. Three 5-μm-thick unstained slides were prepared from each block. A consecutive slide was stained with H&E to assign normal and tumor areas. One pathologist (H. L.) assessed all of the cases. Areas of normal

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[3] The abbreviations used are: LOH, loss of heterozygosity; PSA, prostate-specific antigen; AI, allelic imbalance.

Table 1 *Selected clinical parameters for the 51 prostate cancer patients in the study*

Patient no.	Age at diagnosis (yrs)	Prostate cancer family history	PSA at diagnosis	Pathology stage	Surgical grade
1-612	58	Y[a]	4.3	T_3	7
2-334	65	N	5.9	T_3	7
2-341	66	N	5.5	T_2	6
3-104	63	Y	5.4	T_2	6
3-130	66	N	6.0	T_2	5
3-249	51	Y	5.0	T_2	6
3-342	64	N	5.0	T_3	6
4-188	54	Y	4.6	T_3	7
5-121	55	N	5.7	T_3	7
5-187	63	Y	5.7	T_3	7
5-189	62	N	3.5	T_2	6
5-350	60	Y	6.3	T_2	6
5-369	66	N	5.6	T_2	7
5-436	63	Y	6.0	T_2	6
5-665	65	Y	6.0	T_3	5
5-905	63	N	5.4	T_2	6
6-201	64	Y	5.4	T_2	5
6-322	56	Y	5.6	T_2	6
6-350	58	N	5.6	T_2	7
6-425	61	N	6.2	T_2	6
6-452	64	Y	6.3	T_3	7
7-155	65	Y	5.5	T_3	7
7-187	63	Y	5.3	T_3	7
7-206	71	Y	5.5	T_2	7
7-220	56	Y	6.0	T_2	6
7-286	57	N	7.2	T_3	7
7-293	62	N	5.9	T_2	7
7-297	57	N	10.9	T_3	6
7-309	68	N	4.4	T_2	7
7-310	63	N	7.1	T_2	7
7-311	62	N	6.1	T_2	6
7-324	58	N	8.0	T_2	5
7-341	62	N	12.9	T_3	7
7-348	60	Y	17.0	T_3	7
7-353	56	N	5.9	T_2	7
7-375	57	N	4.8	T_2	6
7-392	60	N	13.0	T_2	9
7-393	58	N	3.4	T_2	7
7-401	59	N	25.0	T_3	7
7-404	60	Y	8.0	T_2	7
7-410	73	N	5.0	T_2	6
7-433	66	N	9.8	T_3	7
7-441	69	N	10.0	T_3	8
7-451	68	N	5.3	T_2	7
7-475	55	Y	6.6	T_2	6
7-484	62	N	8.2	T_2	7
7-485	63	N	7.6	T_2	7
7-491	63	Y	4.3	T_2	7
7-684	48	Y	5.9	T_2	7
7-923	71	Y	5.0	T_3	7
8-501	47	N	18.2	T_3	9

[a] Y, yes; N, no.

and cancer tissue were microdissected with the aid of the outlined H&E-stained slide, as described previously (31, 32). Four patients were removed from the study because the archived tissue did not yield DNA of sufficient quality for PCR amplification. The total number of patients that were included in our genetic analysis was 51.

After microdissection, DNA was extracted using the QiaAmp Tissue Kit (Qiagen, Valencia, CA). The final elution was performed in 100 μl of Tris buffer (pH 9).

Marker Information and Radiation Mapping. Seven microsatellite markers were used in the study. Five of these markers (*D16S3049, D16S3096, D16S516, D16S504,* and *D16S3040*) showed significant linkage in our prostate cancer sibling pair study (15). Sequence information for the seven microsatellite markers used in the present study was obtained from the Genome Database web site.[4] Oligonucleotide primers were synthesized by Genosys Biotechnologies (The Woodlands, TX). An optimal PCR annealing temperature was determined for each microsatellite marker.

The chromosomal order of the microsatellite markers used in the study was confirmed using the Stanford high-resolution TNG3 radiation hybrid panel (Research Genetics, Huntsville, AL). The panel, consisting of DNA from 90 human lymphoblastoid-derived human:hamster hybrids, was screened using each of the seven microsatellite markers. The PCR reactions were performed using a PCR thermal cycler (Ericomp, San Diego, CA). Each 15-μl reaction contained 2 μl of eluted DNA, 1.25 mM of each deoxynucleotide triphosphate, 0.5 μM of each primer, 0.75 unit of Taq DNA polymerase (Life Technologies, Inc., Rockville, MD), 67 mM Tris-HCl (pH 8.8), 6.7 mM magnesium chloride, 16.6 mM ammonium sulfate, 10 mM β-mercaptoethanol, 10% DMSO, and 1× Redi Load (Research Genetics). The step cycle file was comprised of a 5-min denaturation at 94°C and 35 cycles of 94°C for 45 s, annealing at the appropriate temperature for 1 min, and extension at 72°C for 1 min, followed by a final extension of 72°C for 7 min. Ten μl of each PCR reaction were run on a 6% nondenaturing polyacrylamide gel and visualized by ethidium bromide staining. The PCR result of the presence or absence of each marker was recorded for each hybrid, analyzed using the Map Manager QT program (33), and used to determine the overall chromosomal order and the approximate distance between markers.

Allelic Imbalance Studies. For the AI studies, separate PCR reactions were performed using DNA from microdissected normal and tumor tissue. A fluorophore was included at the 5′ end of each forward primer for detection on an ABI Prism 373 XL DNA Sequencer. The fluorophore choice, 6-carboxyfluorescein, tetrachloro-6-carboxyfluorescein, or 4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein, was made to allow for multiplexing of the PCR products for loading. PCR conditions were as described above, but without Redi Load. To determine the appropriate dilution for the genetic analysis, 2 μl of each PCR reaction were run on a 6% nondenaturing polyacrylamide gel and visualized by ethidium bromide staining. The PCR products were diluted in water, with PCR products from a maximum of four markers from one patient multiplexed for loading. One μl of each multiplexed solution was combined with formamide gel loading dye containing 1.5 mM EDTA and 3 mg/ml dextran blue and a 350 base pair 6-carboxytetramethylrhodamine size standard (Perkin-Elmer, Foster City, CA). The sample mixtures were denatured at 95°C for 5 min and immediately put on ice before loading onto a 6% denaturing polyacrylamide gel. Gels were run for a minimum of 7 h at 30 W with ABI Collection software (Perkin-Elmer). ABI GeneScan software version 3.1 (Perkin-Elmer) was used to process the runs. The software package Genotyper version 2.1 (Perkin-Elmer) was used to analyze the data for AI. Alleles were quantitated by peak heights (as recommended by Perkin-Elmer Corp.). The ratio of allele 1 to allele 2 for the tumor was divided by the ratio of allele 1 to allele 2 for the normal sample of each patient. A ratio less than 0.75 or greater than 1.33 was assigned as AI, as described previously (32).

Statistical Analysis. Basic descriptive statistics were used to quantify the evidence for the presence of a tumor suppressor gene at the markers of interest. In addition, contingency table analyses were used to evaluate whether AI at each marker correlated with clinical parameters (*e.g.*, family history of prostate cancer). Test statistics were calculated using Fisher's exact test because some cell counts were small. All analyses were undertaken with the statistical software SAS (SAS Institute Inc., Cary, NC).

RESULTS

Radiation Hybrid Mapping of 16q Microsatellite Markers. The order of the seven microsatellite markers used in this study was analyzed using the Stanford high-resolution TNG3 radiation hybrid panel. All of the markers had been mapped and ordered previously by Genethon (34, 35). We found the mapping order of these markers to agree with the Genethon map, with the exception of marker *D16S3144*. According to our radiation hybrid mapping data, marker *D16S3144* lies distal to marker *D16S504*. Table 2 shows the chromosomal order of the markers based on analysis with Map Manager QT. An intermarker distance could not be obtained for the most proximal markers used in the study (*D16S518* and *D163049*), suggesting that they were too far apart to map with this panel. The estimated physical distance between the markers is shown in Table 2. Markers *D16S3096* and *D16S516*, which were subsequently shown to exhibit the highest frequency of AI, were shown to lie within approximately 118 kb, based on our radiation hybrid mapping results.

[4] http://gdbwww.gdb.org.

Allelic Imbalance of 16q Markers. Fifty-one prostate cancer samples were screened for AI using a panel of seven microsatellite markers on chromosome 16q23. The overall frequency of AI with each marker is shown in Table 3. The markers that showed the highest frequency of AI were *D16S3096* (45%) and *D16S516* (53%). The most distal marker, *D16S3040,* showed the lowest frequency (14%) of AI, and this may reflect background instability. Twenty-eight of the 51 (55%) samples studied showed AI involving *D16S3096* and/or *D16S516,* and interstitial AI involving one or both of these two markers was seen in 19 of the 51 (37%) samples. We define interstitial AI in this study as prostate tumors that show genomic deletions within the region defined by the five internal markers used in the study (*D16S3049, D16S3096, D16S516, D16S504,* and *D16S3144*). The patterns of AI for these 19 samples are shown in Fig. 1. Representative histograms for two of the samples showing interstitial imbalance within this region are shown in Fig. 2.

Clinical Associations. Potential clinical associations with the presence of AI at each marker were assessed. Clinical parameters examined included age, family history, surgical Gleason grade, PSA at diagnosis, and pathological T stage. No noteworthy associations were identified when all 51 samples were examined. However, we also examined any associations between those samples that showed interstitial AI involving markers *D16S3096* and *D16S516* (from Fig. 1) compared with seven samples that showed no AI at either marker. This analysis was performed to eliminate any confounding due to adjacent regions of AI. Here, we observed a significant association between family history and the presence of AI at markers *D16S3096* and *D16S516* ($P = 0.048$). Eight of the 19 samples (42%) exhibiting AI at either marker had a positive family history of prostate cancer in at least one first-degree relative. In contrast, none of the seven samples without AI at either marker had a family history of prostate cancer. If samples 5-905, 7-451, and 7-485 are removed from the analysis because they were not informative at one or both markers, this relationship did not change ($P = 0.048$).

Table 2 *Radiation hybrid mapping of seven microsatellite markers on 16q*

Marker	Intermarker distance (cR)[a]
D16S518	[b]
D16S3049	72.46
D16S3096	39.26
D16S516	79.58
D16S504	106.29
D16S3144	76.21
D16S3040	

[a] 1 cR = 3 kb.
[b] D16S518 and D16S3049 were too far apart to map with this panel. Map distances were based on D16S3049 as 0.

Table 3 *AI at chromosome 16q23 loci in 51 primary prostate tumors*

Marker	Primer name	AI/informative samples (percentage of AI)	Cytogenetic location
D16S518	AFMa132xg9	16/42 (38%)	16q23.1-16q24.2[a]
D16S3049	AFMa245yf5	14/39 (36%)	16pter-qter[a]
D16S3096	AFMb322wb9	15/33 (45%)	16q23.2[b]
D16S516	AFM350vd1	20/38 (53%)	16q23.2[b]
D16S504	AFM292xh5	10/29 (34%)	16q23.2[b]
D16S3144	AFM126yb8	7/23 (30%)	16q23[a]
D16S3040	AFMa204xd9	6/43 (14%)	16q23.2[b]

[a] According to the Genome Database (http://gdbwww.gdb.org) and Ref. 34.
[b] According to Los Alamos Laboratories (http://www-ls.lanl.gov/).

DISCUSSION

A goal of this study was to provide further evidence for a prostate cancer susceptibility locus on chromosome 16q23. Our previous genome-wide genetic linkage analysis of 504 sibling brothers affected with prostate cancer showed significant linkage to five genomic regions, with the strongest association on chromosome 16q23 (15). On 16q23, five consecutive markers (*D16S3049, D16S3096, D16S516, D16S504,* and *D16S3040*) showed significant linkage, covering a distance of approximately 7.5 cM. The strongest association was with marker *D16S3096* (15). We argued that the identification of increased AI in prostate tumors using the same markers as those used in the linkage studies would provide further support for a prostate cancer susceptibility gene on chromosome 16q23. Our analysis of 51 primary prostate tumors not only revealed a high AI within this region, but also helped to localize the candidate region to approximately 118 kb.

The highest frequency of AI was found with markers *D16S3096* (45%) and *D16S516* (53%) that map to chromosome 16q23.2. Previous studies that examined AI at 16q23.2 in prostate tumors reported frequencies that varied between 23% and 56% (17, 18, 20, 21, 25). Studies that used samples with greater than 50% tumor involvement (similar to our study) reported 48–56% AI in this region (18, 21, 25), consistent with our findings. Furthermore, we found that 37% (19 of 51) of prostate tumors showed interstitial AI involving markers *D16S3096* and/or *D16S516.* In all of these tumors, deletions were restricted between the most distal and proximal markers used in the study. These data suggest that we have precisely localized a candidate prostate cancer tumor suppressor gene between markers D16S3096 and *D16S516.* We estimate the distance between markers *D16S3096* and *D16S516* to be approximately 118 kb using the Stanford high-resolution TNG3 radiation hybrid panel. This estimate of distance between these two markers is supported by the fact that we have

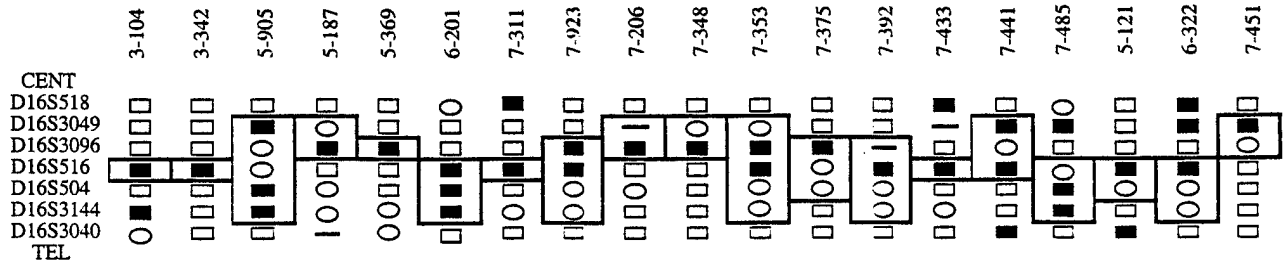

Fig. 1. Specific loss on 16q involving D16S3096 and/or D16S516 in 19 primary prostate tumors. Summary of samples that showed interstitial AI. Sample numbers are listed across the *top*; markers are listed on the *left*. □, informative samples with no AI; ■, informative samples with AI; *ovals*, noninformative (homozygous) samples; *dashes*, no result. The maximum area of AI is *boxed* for each sample.

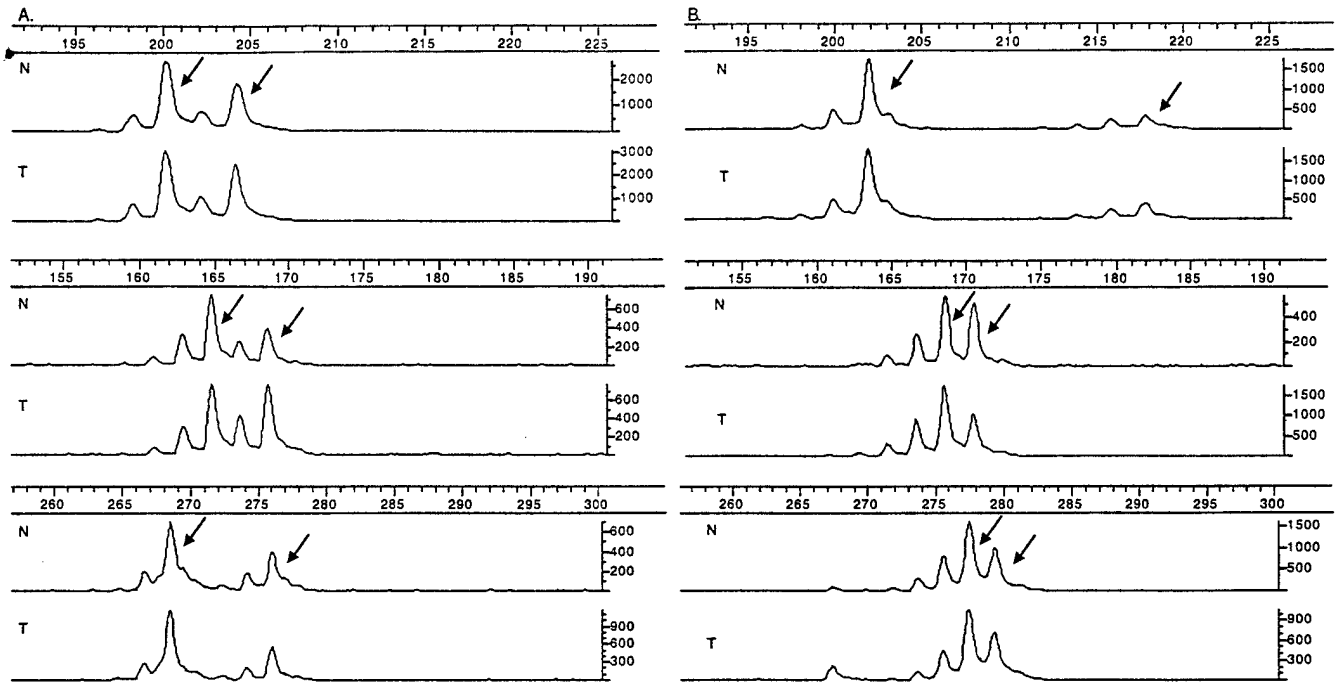

Fig. 2. Examples of histograms from AI studies on normal (N)/tumor (T) prostate cancer pairs. *A*, sample 3-104. The *top panel* shows no AI for marker D16S3096 (T:N ratio = 0.854), the *middle panel* shows AI for marker D16S516 (T:N ratio = 0.517), and the *bottom panel* shows no AI for marker D16S504 (T:N ratio = 1.189). *B*, sample 7-433. The *top panel* shows no AI for marker D16S3096 (T:N ratio = 0.856), the *middle panel* shows AI for marker D16S516 (T:N ratio = 1.496), and the *bottom panel* shows no AI for marker D16S504 (T:N ratio = 0.911). *X* axis, size (in bp); *Y* axis, fluorescence intensity (in arbitrary units); *arrows*, the alleles.

subsequently identified four bacterial artificial chromosome clones that are positive for both markers.[5]

At least three loci on chromosome 16q have been reported to be involved or deleted in prostate tumors, including 16q23 (17, 18, 25). In a study of 59 prostate tumors, Latil *et al.* (4) reported an AI frequency of approximately 50% with markers D16S518 and D16S507 on 16q23.2, a region they estimated to be 10 cM. This region encompasses markers D16S3096 and D16S516 that define our region of AI. A commonly deleted region on 16q23–24 defined by markers D16S515 and D16S516 was also reported in prostate tumors by LOH studies (25). This region also encompasses markers D16S3096 and D16S516 but may extend more proximal to these markers, where another region of AI in prostate tumors has been mapped. A third study of LOH in prostate tumors also implicates 16q23.2–24.1, but once again, the region defined extends distal to 16q23.2 and may encompass a region of AI implicated in prostate cancer metastasis (17). Therefore, we have precisely mapped a region of AI in prostate tumors that may have been reported previously in independent studies.

We did not identify noteworthy associations between any of the clinical parameters examined and the presence or absence of AI at any marker when all of the samples were included in the analysis. However, we did identify a statistically significant association between AI at markers *D16S3096* and *D16S516* and family history of prostate cancer when comparing those samples that showed interstitial AI involving these two markers with those without any evidence for AI with these markers. These restricted analyses were undertaken to eliminate potential confounding due to possible overlapping genomic deletions corresponding to adjacent distal and proximal prostate cancer loci. In conjunction with our independent sibling pair linkage analyses that identified this region (and marker *D16S3096* in particular), these findings strongly suggest that this region may harbor a prostate cancer tumor suppressor gene involved in both nonfamilial and hereditary forms of prostate cancer.

If independent studies support our findings, this would suggest that a gene in this region may be analogous to the gene *APC*, which is the familial adenomatous polyposis colon cancer susceptibility gene (36), as well as being implicated in the development of the majority of colorectal cancers (37). LOH corresponding to chromosome 16q23 has also been reported in breast cancer and other cancers (26–28); therefore, this locus may harbor a tumor suppressor gene that is inactivated in many different cancers. No tumor suppressor genes have been reported to be mapped to the region that we have identified. Interestingly, two recent publications report that this region contains a fragile site, FRA16D (38, 39). Fragile sites are thought to be more prone to breaks; therefore, a neighboring tumor suppressor gene may be deleted as a result of such breakage.

In summary, we have identified a high frequency of AI in prostate tumors on chromosome 16q23.2 localized to two markers, *D16S3096* and *D16S516*. This locus corresponds to a region that we identified previously in our prostate cancer sibling pair linkage analyses (15). Taken together, these data suggest that we have precisely localized a region of chromosome 16q23.2 that harbors a prostate cancer tumor suppressor gene implicated in the development of both nonfamilial and familial forms of prostate cancer cases.

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[5] P. L. Paris and G. Casey, unpublished data.

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